Glycosyl Bromides in Glycoside Synthesis: Development of New Promoter System and Metal-Mediated Regioselective Glycosylations

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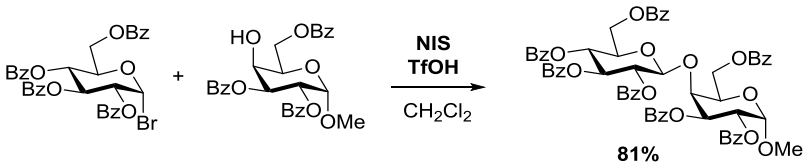
Abstract

Investigation of Promoter Systems for Efficient Activation of Glycosyl Halides

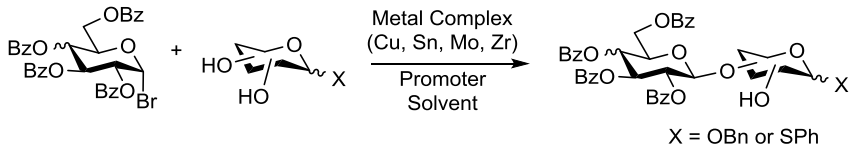
New efficient procedures for activation of glycosyl halides are in high demand. The Koenigs-Knorr reaction has been the paramount coupling reaction between saccharides for more than a century. However in this and related procedures glycosyl halides are generally activated by toxic and expensive metals salts, which most commonly contain silver or mercury.



As a result, iodonium ions have been investigated for activating disarmed glycosyl bromides in the glycosylation of glycosyl acceptors. A method employing iodonium ions generated from *N*-iodosuccinimide and a protic acid was developed. The best results were obtained with the benzoyl protected glycosyl donors and acceptors. This method allows for the use of highly disarmed glycosyl bromides in a metal free glycosylation.

Metal-mediated Regioselective Coupling of Unprotected Carbohydrate Acceptors

A straightforward procedure for regioselective glycosylation of unprotected glycosyl acceptors using metal complexes was investigated in this project. This investigation was conducted using disarmed glycosyl bromides and unprotected glycopyranoside acceptors.



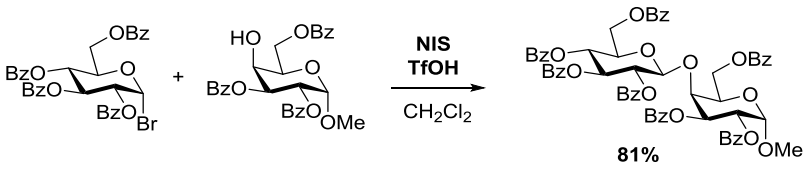
Phenyl 1-thio-D-glycopyranosides and benzyl D-glycopyranosides derived from D-glucose, D-galactose and D-mannose were treated with various metal complexes. Treatment with copper complexes followed by glycosylation did not yield any desired disaccharide products, however for tin, molybdenum and zirconium selective glycosylation of either the 3 or the 6 position was achieved. Acceptors derived from D-galactose underwent regioselective glycosylation of the 3 position in 51% yield employing dibutyltin dichloride.

Molybdenum dioxide complexes facilitated a regioselective glycosylation of the 6 position for acceptors derived from D-glucose and D-mannose in 52% and 35% yield, respectively. Zirconium complexes achieved the best results with benzyl D-mannopyranoside acceptors resulting in 40% yield of the 1,6-linked product.

Resumé

Undersøgelse af promoter systemer for effektiv aktivering af glykosyl halider

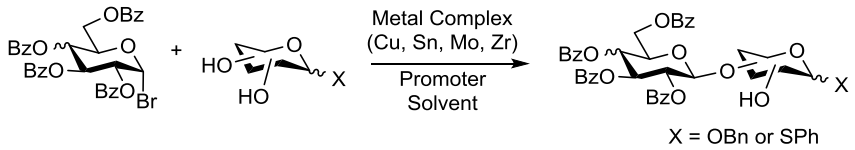
Effektive procedurer for aktivering af glykosyl halider er yderst efterspurgte. Koenigs-Knorr reaktionen har været en altafgørende koblingsreaktion mellem sakkarider i mere end et århundrede. I denne og lignende procedurer bliver glykosyl halider generelt aktiveret af giftige og dyre metalsalte, som almindeligvis indeholder sølv eller kviksølv.



Som følge, er iodonium ioner blevet undersøgt til aktivering af ureaktive glykosyl bromider i glykosyleringen af glykosyl acceptorer. En metode med iodonium ioner genereret fra *N*-iodosuccinimide og en protisk syre blev udviklet i denne afhandling. De bedste resultater blev opnået med benzoyl beskyttede glykosyl donorer og acceptorer. Denne metode tillader brugen af ureaktive glykosyl bromider i glykosylering uden tungmetaller.

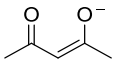
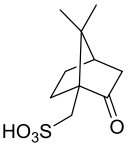
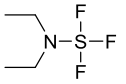
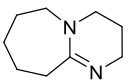
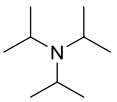
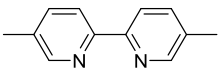
Metal-medieret regioselektiv kobling af ubeskyttede kulhydrat-acceptorer

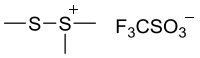
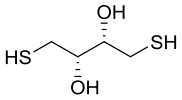
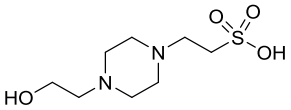
En ligefrem procedure til regioselektiv glykosylering af ubeskyttede glykosyl acceptorer med metalkomplekser blev undersøgt i dette projekt. Undersøgelsen blev udført med ureaktive glykosyl bromider og ubeskyttede glykopyranosid acceptorer.

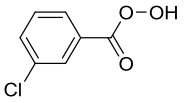
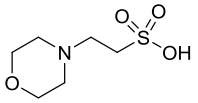
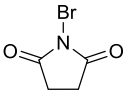
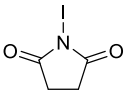
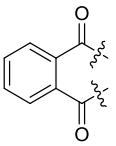
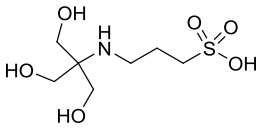


Phenyl 1-thio-D-glykopyranosid og benzyl D-glykopyranosider afledt D-glukose, D-galaktose and D-mannose blev udsat for forskellige metal komplekser. Behandling med kobberkomplekser blev efterfulgt af glykosylering, hvilket ikke resulterede i nogle af de ønskede disakkarid produkter. Behandlingen med tin, molybdenum and zirconium efterfulgt af glykosylering resulterede i selektiv glykosylering af enten 3 eller 6 positionen. En acceptor tilhørende D-galaktose familien blev regioselektiv glykosyleret i 3 positionen i 51% udbytte ved brug af dibutyltin dichlorid. Molybdenum dioxid komplekser muliggjorde en regioselektiv glycosylering af 6 positionen for acceptorer tilhørende D-glukose- and D-mannosesukrene i henholdsvis 52% og 35% udbytte. Zirconium komplekser opnåede det bedste resultat med benzyl D-mannopyranosid acceptoren, hvilket resulterende i det 1,6-koblede disakkarid med udbytte på 40%.

Abbreviations

Ac	Acetyl
acac	acetylacetonato 
AceA	L-Aceric Acid
Api	D-Apiose
Ara	L-Arabinose
AW	Acid washed
Bn	Benzyl
Bu	Butyl
Bz	Benzoyl
COSY	Correlation Spectroscopy
Cp	cyclopentadienyl 
CSA	Camphor-10-sulfonic acid 
DAST	(diethylamino) sulfur trifluoride 
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene 
DCE	1,2-Dichloroethane
Dha	3-Deoxy-D-lyxo-hept-2-ulopyranosaric acid
DIPEA	*N,N*-Diisopropylethylamine 
DMBPY	5,5′-Dimethyl-2,2′-dipyridyl 

DME	Dimethoxyethane
DMF	*N*,*N*-dimethylformamide
DMSO	Dimethyl sulfoxide
DMTST	Dimethylthiomethylsulfonium triflate 
DTT	Dithiothreitol 
Et	Ethyl
Fuc	L-Fucose
Gal	D-Galactose
GalA	D-Galacturonic acid
Glc	D-Glucose
GlcA	D-Glucuronic acid
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid 
HG	Homogalacturonan
HMBC	Heteronuclear multiple bond correlation
HMPA	Hexamethylphosphoramide
HPLC	High pressure liquid chromatography
HRMS	High resolution mass spectroscopy
HSQC	Heteronuclear single quantum coherence
IDCP	Iodonium di-*sym*-collidine perchlorate
KDO	3-Deoxy-D-manno-oct-2-ulosonic acid
Lev	Levulinoyl

mCPBA	*meta*-Chloroperoxybenzoic acid	
Me	Methyl	
MES	2-(*N*-morpholino)ethanesulfonic acid	
MS	Molecular sieves	
LG	Leaving Group	
NBS	*N*-bromosuccinimide	
NIS	*N*-iodosuccinimide	
NMR	Nuclear magnetic resonance	
Pent	4-Pentenyl	
PG	Protecting group	
Ph	Phenyl	
Phth	Phthaloyl	
RG	Rhamnogalacturonan	
Rha	L-Rhamnose	
TAPS	3-[[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]amino]propane-1-sulfonic acid	
TBAB	Tetrabutylammonium bromide	
TBAF	Tetrabutylammonium fluoride	
TBAI	Tetrabutylammonium iodide	

TBDMS *tert*-Butyldimethylsilyl

TBDPS *tert*-Butyldiphenylsilyl

TBS Tributylsilyl

TES Triethylsilyl

Tf Trifluoromethanesulfonyl

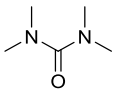
TFA Trifluoroacetic acid

THF Tetrahydrofuran

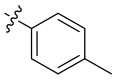
TLC Thin layer chromatography

TMS Trimethylsilyl

TMU Tetramethylurea

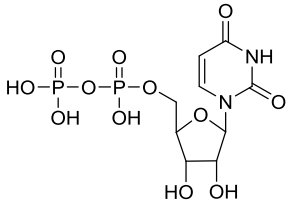


Tol Tolyl



Troc 2,2,2-Trichloroethyl carbonate

UDP Uracil diphosphate



Xyl D-Xylose

Preface

This thesis encompasses the work carried out during my PhD studies at the Technical University of Denmark (DTU) from December 2012 to April 2016 under the supervision of Professor Robert Madsen. During this time I had the opportunity to work with different aspects of glycosylation chemistry, as well as plant cell wall biosynthesis. The thesis describes my work regarding the development of iodonium ions for efficient activation of glycosyl bromides and investigations of metal-mediated regioselective glycosylation of unprotected carbohydrate acceptors. Lastly, a chapter on the work conducted during my external stay at the Joint BioEnergy Institute (JBEI) is included. A publication regarding the development of a new promoter system is submitted and accepted by European Journal of Organic Chemistry.

My PhD studies have been highly rewarding both in regard to me becoming a more accomplished scientist and personal development. Many people have contributed to this amazing journey. First and foremost I would like express my profound gratitude to my supervisor professor Robert Madsen, for the opportunity to become part of his research group and an always open door, patience and guidance whenever needed.

I am thankful to both past and present members of the Madsen group, it has been a privilege working with all of you. I would especially like to thank my lab mate during all of it, Dominika Niedbal for discussions about sugar, chemistry and all other matter of life – I will miss your company a lot.

I would like to thank the technical staff of the department for superb assistance and support. Especially I would like to thank Anne Hector for never-ending help with acquisition of NMR data. Also, I want to thank everyone from the organic chemistry section of DTU Chemistry.

I am grateful to professor Henrik Vibe Scheller for hosting me at JBEI and postdoctoral fellow Solomon Stonebloom for help with the project. Also, I would like to thank the students contributing to my projects:

Christine Thue Poulsen, Ida Slot Arakelian Jensen and Mette Camilla Lindén. I am grateful to Niels Storm Knigge and Ragnhild Ohm for proofreading my thesis.
I want to thank my friends for cheering me on. Most of all I want to express my deepest gratitude and thanks to Naram El-Shamary and Christian Lanz for endless support and encouragement. It has made a world of difference to me.
Lastly, I want to thank the Danish Research Council for Independent Research - Natural Sciences for financial support, as well as Christian og Ottilia Brorsons Rejselegat and Oticon Fonden for financial support of my external stay.

Table of Contents

1 Introduction

Carbohydrates and glycoconjugates of varying complexity are of crucial importance to numerous biological processes. Varki stated in 1993 "all of theories are correct"[1] relating to discussions of the functions of carbohydrates in the field of glycobiology. Included in a wide array of functions pertaining to oligosaccharides are conformation, half-life and stability of proteins, antibodies, toxins, cell signaling and purely structural properties. Apweiler *et al.*[2] estimated in 1999 based the SWISS-PROT database that more than half of naturally occurring proteins in humans are likely to be glycosylated. Alterations in glycosylation patterns in many different proteins have been shown to result in change or loss of function for a given type of protein, underscoring the importance of carbohydrates in biological systems.[1]

An increasing need for a better understanding of these biological processes has resulted in a growing demand for well-defined and pure carbohydrates and glycoconjugates. Chemical synthesis of oligosaccharides offers the flexibility to generate both well-defined natural oligosaccharides and artificial structures as a result of functional group manipulation. In addition, chemical synthesis provides a way of gaining larger quantities of pure oligosaccharides.[1,3,4]

1.1 Aspects of Glycosylation Chemistry

The chemical synthesis of oligosaccharides represents a number of challenges, which most often are specific to a given carbohydrate structure in regard to stereo- and regioselectivity. The complexity of the formation of the glycosidic bond can be illustrated with just two glucose molecules, since these together can form 11 different glycosidic linkages (Figure 1). Many strategies and methods have been developed to overcome these challenges, but general applicability remains lacking, and as a result the

quest to develop and reinvent the field of carbohydrate chemistry continues.

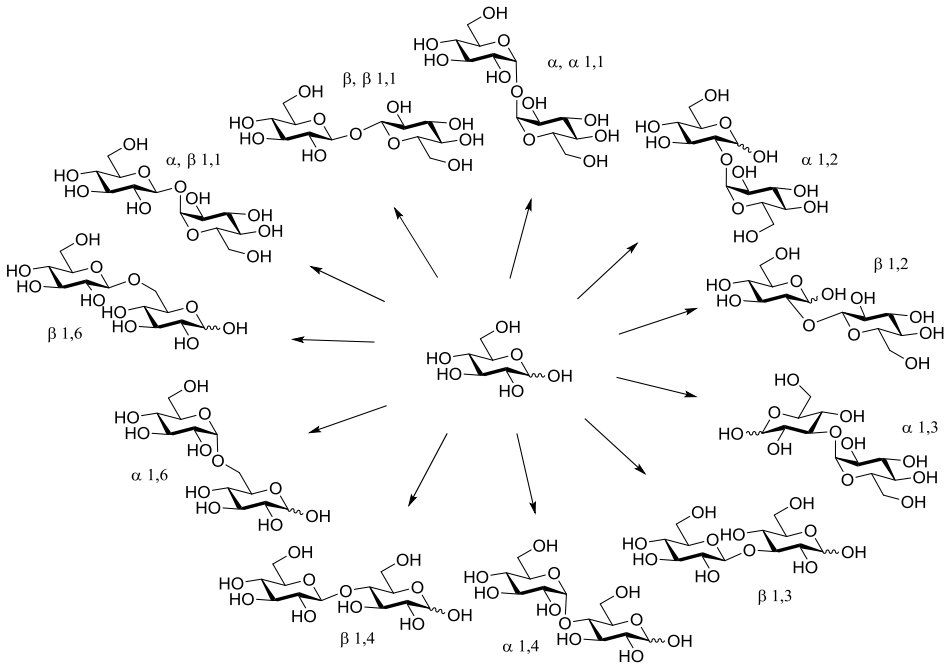
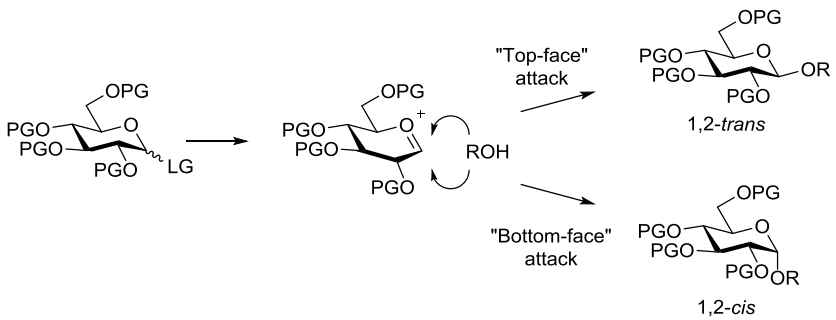


Figure 1 All possible glycosidic linkages between two glucose molecules.

The most important challenges are the stereochemical control in formation of the glycosidic linkage and differentiation between the multiple functional groups of similar character in pyranoses. Formation of the glycosidic bond is done by activation of the leaving group (LG) of the glycosyl donor, hereby forming the oxocarbenium ion[5]. Scheme 1 illustrates this activation with a glucopyranosyl donor. The oxocarbenium ion of the glucopyranosyl donor is attacked by a nucleophile from either the "top-face" or the "bottom-face", which results in the 1,2-*trans* or the 1,2-*cis* glucoside, respectively.

The stereochemical outcome of the anomeric position is influenced in several ways, such as sterics, anomeric effect, choice of protecting groups and solvent.



Scheme 1 General glycosylation: Activation of the glycosyl donor leading to formation of the oxocarbenium ion, which is attacked from either the top face or the bottom face leading to the 1,2-*trans* or the 1,2-*cis* glycoside, respectively.

Regioselectivity in the glycosylation is most often achieved by blocking the hydroxyl groups that should not participate in the glycoside formation. The most common protecting groups employed for hydroxyl functionalities in glycosylation chemistry are ethers, esters and acetals, which are further grouped into temporary and permanent groups depending on their lability and ability for selective removal. Some examples of frequently used protecting groups are shown in Figure 2.

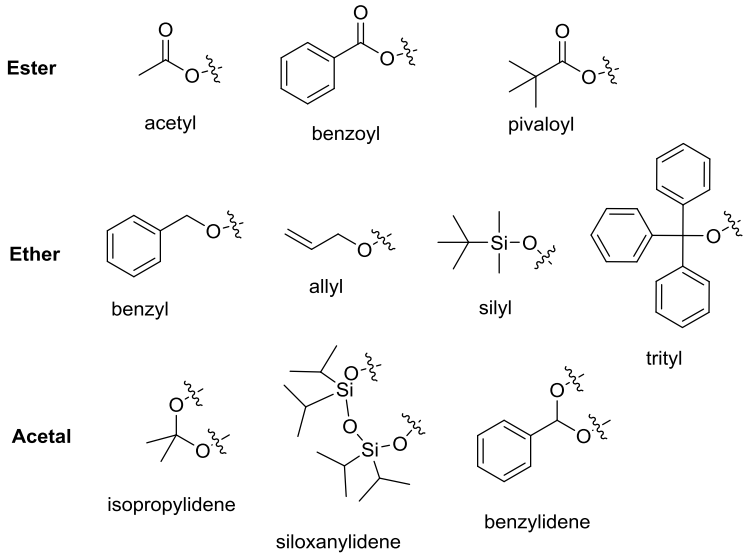
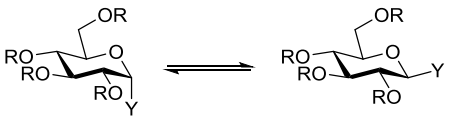


Figure 2 Ester, ether and acetals are the most commonly employed types of protecting groups in carbohydrate synthesis.

1.1.1. Anomeric Effect

The anomeric effect plays a role in the stereochemical outcome of reactions at the anomeric center. For steric reasons equatorial substituents on pyranoses in the $^4C_1$ conformation are thermodynamically favored. The anomeric substituents are an exception to this rule, since they are also influenced by the anomeric effect, which results in anomeric mixtures. An example hereof is D-glucose which exists as 64% β-anomer and 36% α-anomer in aqueous solution. This phenomenon, opposing general observations was discovered by Lemieux[6,7] who coined it the anomeric effect. The anomeric effect was first observed for glycopyranosides and later established as a general trend for tetrahedral carbon centers carrying two or more heteroatoms. This behavioral trend is defined as the generalized anomeric effect for molecules containing a C-X-C-Y moiety, where X = O, S or N and Y = Br, Cl, F, N, S or O as shown in Scheme 2. The influence of the anomeric effect is associated with the electronegative nature of Y. The more electronegative a substituent the greater the impact of the anomeric effect.[8]



Scheme 2 Influence of the electronegativity of Y on the anomeric configuration. The ratio of axial anomeric substituent increases with a more electronegative Y and the ratio of equatorial to anomeric substituent increases when Y is less electronegative.

The explaination for the anomeric effect is related to the dipole-dipole interaction and the stereoelectronic effect. Both theories are based on the importance of the nonbonding electron pairs of the endocyclic oxygen, which form a dipole in a direction away from the ring. Another dipole is formed from the anomeric carbon to the heteroatom of the substituent. For a D-sugar with an α-substituent, the dipoles are in opposite direction from the endocyclic oxygen, which is energetically favored due to a small dipole-dipole interaction, compared to a larger interaction created by the

β-substituent being parallel to the dipole of the endocyclic ring oxygen (Figure 3).

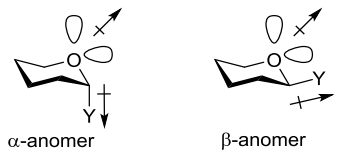


Figure 3 Illustration of dipole moments for α and β anomers in $^4C_1$ configuration.

In the stereoelectronic version of the anomeric effect the α-substituted D-sugar is favored energetically, because the nonbonding electron pair placed in the axially oriented sp^3-orbital of the endocyclic oxygen overlaps with the antibonding orbital of the anomeric substituent. This overlap is not possible for the corresponding β-substituted D-sugar, which is depicted in Figure 4.

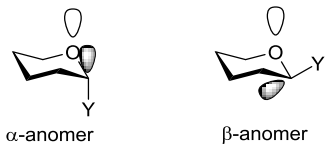


Figure 4 Orientation of orbitals for α and β anomers in $^4C_1$ configuration.

The exo-anomeric effect can be explained in a comparable manner with stereoelectronic effects by an overlap between the antibonding orbital of C_1-O_{Endo} bond with one of the nonbonding electron pairs of the exocyclic oxygen.[8]

1.1.2. 1,2-*trans*: Neighboring Group Participation

The general tools to control the stereoselective formation of the glycosidic bond are to a large extend comprised of solvent effect and neighboring group participation. The latter is achieved through an ester functionality in the 2-position, which forms an acetoxonium ion effectively blocking the bottom face of the glycopyranoside after departure of the leaving group.

The nucleophilic attack of an acceptor on the acetoxonium ion can only occur from the "top face", hence resulting in the 1,2-*trans* glycoside as shown in Figure 5, and in this way translates into the β-glucosides, β-galactosides and α-mannosides respectively.

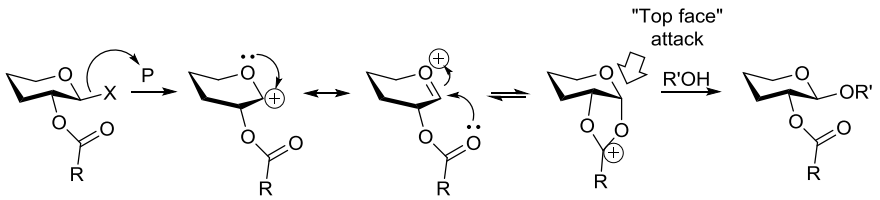


Figure 5 Neighboring group participation leading to 1,2-*trans* glycoside.

1.1.3. 1,2-*cis*: *in situ* Anomerization

Formation of the 1,2-*cis* glycosides cannot be achieved in the same straight forward manner.[9] The reactivity of glycosyl donors are very much influenced by the protecting groups applied, which will be described later in more detail in section 1.3. 1,2-*Cis* glycosides are formed through the oxocarbenium ion which allows for nucleophilic attack from both faces, and thereby also giving rise to simultaneous formation of the 1,2-*trans* glycoside resulting in an anomeric mixture. Essential for the formation of 1,2-*cis* glycosides is a non-participating group in the 2-position, such as ether protecting groups.

Benzyl protected glycosyl bromides are highly reactive, which can be exploited to form 1,2-*cis* glycosidic linkages. For the reactive benzylated glycosyl bromides Lemieux and co-workers[10] developed *in situ* anomerization as shown in Figure 6, which is promoted by tetraalkyl ammonium bromide and Hünig's base.[10] This type of activation is mild and provides excellent 1,2-*cis* stereoselectivity. However the scope of this method is limited to reactive substrates only, including the acceptors.

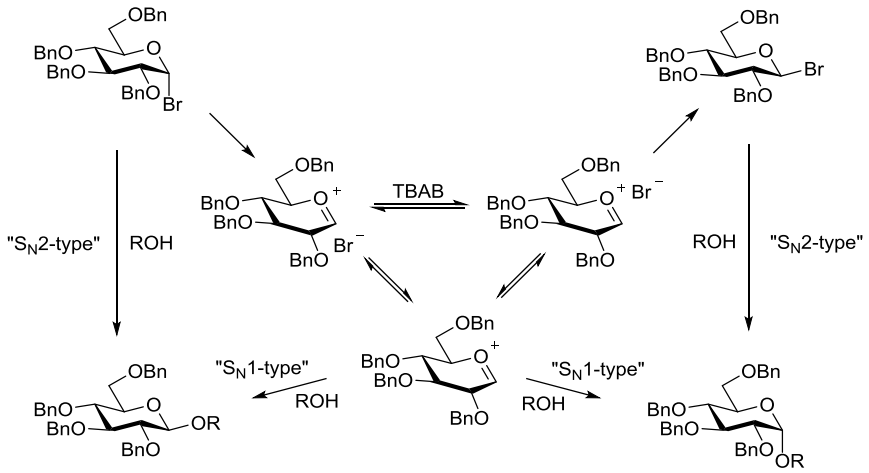


Figure 6 Lemieux's *in situ* anomerization. The axial glycosyl bromide **(X)** dissociates and forms a tight ion pair between the oxocarbenium ion and the bromine ion, which rearranges in the precense of TBAB, hereby allowing access to the equatorial glycosyl bromide **(X)** and the 1,2-*cis* glycoside through an "SN2-type" reaction.

In situ anomerization allows 1,2-*cis* glycosides to be formed as a result of a S_N2-type reaction on the anomeric position of a β-glycosyl bromide. 2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranosyl bromide is formed *in situ* from 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide with tetraalkyl ammonium bromide as illustrated in Figure 6. 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl bromide dissociates into the oxocarbenium ion, which forms a tight ion pair with the bromine ion in an α-configuration. The α-configuration can be shifted into the β-configuration using tetrabutylammonium bromide (TBAB) resulting in 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl bromide. The *in situ* formed β-glucopyranosyl bromide is highly reactive and will therefore instantly react with an alcohol acceptor in a S_N2-type fashion resulting in the 1,2-*cis* product.

1.1.4. Solvent Effect

In a S_N1-type reaction between a nucleophile and an oxocarbenium ion the stereoselectivity can be influenced by the choice of solvent. Solvent effects have been established for both formation of 1,2-*trans* and 1,2-*cis* glycosidic

linkages as shown in Figure 7. Participation of ether solvents can form an equatorial oxonium ion, leaving the bottom face open for nucleophilic attack, hence creating a preference for the 1,2-*cis* glycoside. On the other hand acetonitrile forms an axial α-nitrilium ion with the oxocarbenium ion blocking the bottom face, which has been coined the nitrile effect.[11,12]

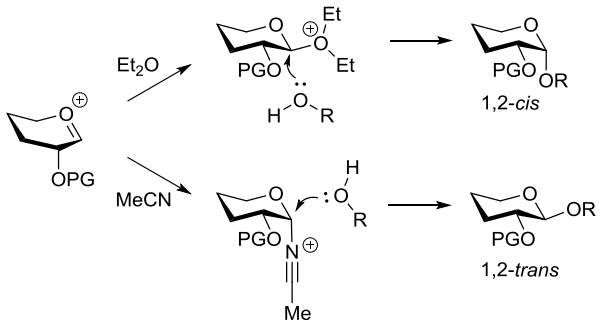


Figure 7 Influence of ether and acetonitrile solvents on formation of the glycosidic linkage.

1.2 Glycosylation Methods

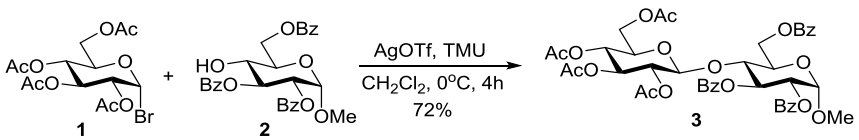
Different anomeric leaving groups can be employed to form the oxocarbenium ion. These leaving groups will react with a suitable promoter and break the bond to the anomeric center, hence forming the oxocarbenium ion or the acetoxonium ion for subsequent reaction with the nucleophile. The most frequently employed glycosyl donors are glycosyl halides, thioglycosides, glycosyl trichloroacetimidates and pentenyl glycosides. Since the projects presented here are pertaining to glycosyl bromides, the focus will be on the glycosyl halides.

1.2.1. Glycosyl Halides

Great efforts have been put into the development of new glycosyl donors, but even so the classical Koenigs-Knorr glycosylation with glycosyl halides

has been of paramount importance in forming glycosidic linkages for more than a century. A glycosyl bromide was originally used in the Koenigs-Knorr glycosylation[10,26], but these are not the only glycosyl halides employed as leaving groups. The other glycosyl halides used include chlorides, fluorides and iodides, differing widely in their reactivity, and therefore also applicable to different situations. Glycosyl chlorides, bromides and iodides are commonly axial at the anomeric center, due to the anomeric effect. The equatorial counterparts are most commonly isolated for less reactive glycosyl fluorides and chlorides.

Glycosyl chlorides and bromides are often grouped together, since they exhibit similar reactivity and stability, which in terms are greatly influenced by protecting groups. In the original publication by Koenig and Knorr[13] from 1901 the acetylated glycosyl bromide was activated using silver(I) carbonate, which is still employed today, along with silver(I) oxide[14]. Hanessian and Banoub[16] employed silver(I) triflate instead of silver(I) carbonate in the Koenigs-Knorr glycosylation. Application of silver(I) triflate became the most widespread modification of the Koenigs-Knorr reaction and is also today widely employed. Silver(I) triflate is highly efficient and generally applicable, which Hanessian and Banoub[16] demonstrated in the initial publication, as shown in Scheme 3 for acetobromoglucose **(1)** as the donor. Furthermore, Silver(I) triflate is soluble in solvents such as dichloromethane, contrary to silver(I) carbonate, silver(I) oxide and other silver(I) salts (*vide infra*).

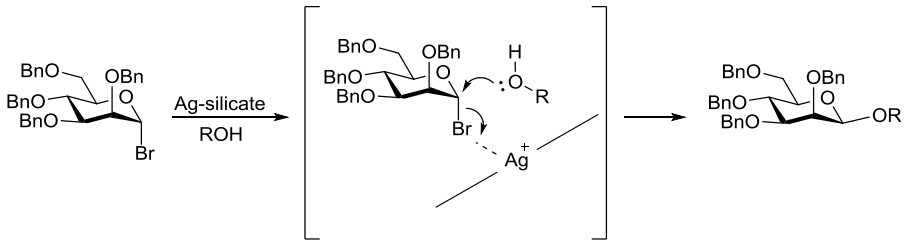


Scheme 3 Initial application of silver(I) triflate by Hanessian and Banoub.[16]

Helferich *et al.*[17–19] introduced mercury salts ($Hg(CN)_2$ and $HgBr_2$) as promoters instead of the aforementioned silver salts for glycosyl bromide activation, which are especially useful for the unreactive substrates. Later on, other heavy metal salts have found common application as promoters

for glycosyl bromides. These include silver imidazolate[20], HgO[21], HgI_2[22], $CdCO_3$[23] and $AgClO_4$[24–27].

Insoluble silver salts are used to create the difficult β-mannosides by reacting the axial glycosyl bromide with the silver surface as shown in Scheme 4, which in turn shields the bottom face by making only the top face available for reaction.[28–33]



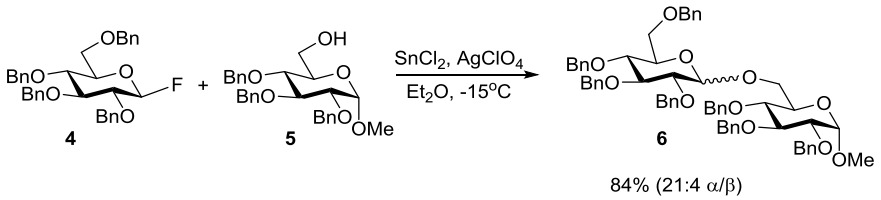
Scheme 4 Application of glycosyl bromides in β-mannoside synthesis with silver silicate as promoter.[34]

Different Lewis acids have been used to activate glycosyl halides as well, but did not cement their applicability in the same manner as the heavy metal salts. These include $SnCl_4$[35], $BF_3 \cdot Et_2O$[35], $ZnCl_2$[36], $ZnCl_2$-$TrCl$[36], $Sn(OTf)_2$[37,38], $Cu(OTf)_2$[39] and $InCl_3$[40]. Activation of glycosyl halides with Tf_2O[41] and $TfOH$[4243] has also been reported, although moderate yields were obtained with the latter. Iodine[44] was used with glycosyl bromides and simple alcohol acceptors resulting in good yield.

The Lemieux *in situ* anomerization[10] (*vide supra*) is another example where the glycosyl bromides are still relevant in carbohydrate synthesis today. Furthermore, glycosyl bromides are having a renaissance in regioselective glycosylation (*vide infra*).

Mukaiyama *et al.*[45] developed glycosyl fluorides as donors activated by $SnCl_2$-$AgClO_4$, as shown in Scheme 5 with the benzyl protected glucopyranosyl fluoride **4**, which are significantly less reactive than their chloride and bromide counterparts, and therefore have an increased shelf-life. However, this increased stability also means that promoters frequently used in the Koenigs-Knorr glycosylation are generally not applicable. Suzuki and co-workers[46,47] developed Cp_2ZrCl_2-$AgClO_4$ and

Cp_2HfCl_2-$AgClO_4$, which together with $SnCl_2$-$AgClO_4$ are the most commonly employed for activation of glycosyl fluorides today. Other activation conditions include different triflates ($Sn(OTf)_2$[48,49], $Cu(OTf)_2$[39], $Yb(OTf)_3$,[50–52], TMSOTf[53] and TfOH[42,43], and Lewis acids such as $BF_3 \cdot Et_2O$[54–56] and $SnCl_2$ in combination with other metal salts[57–59].



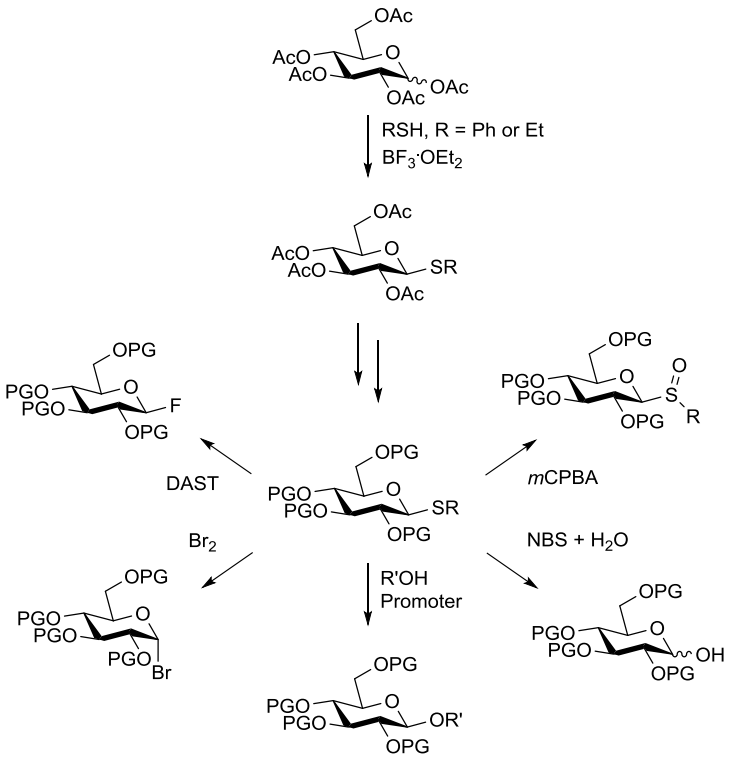
Scheme 5 Mukaiyama and co-workers[45] application of glycosyl fluorides with carbohydrate acceptor.

Glycosyl iodides have been known longer than the glycosyl fluorides.[60] Even so, their applicability has only in recent years been improved. *In situ* formation was used in the early days of glycosyl iodides, due to stability issues of these reactive donors.[61] Improved methods for preparation of glycosyl iodides have made application more appealing.[62–66] Benzyl protected glycosyl iodides are highly reactive and unstable, but the less reactive acyl protected glycosyl iodides have been prepared and characterized.[66] Glycosyl iodides can be activated with either a base[67] or they can participate in a halide-ion catalyzed glycosylation[68–72] similar to Lemieux *in situ* anomerization (*vide supra*).

1.2.2. Thioglycosides

Thioglycosides were first investigated by Ferrier and co-workers[73] as glycosyl donors, where mercury(II) acetate as employed for activation to form both α- and β-glucosides in good yield. Since then, thioglycosides have found wide application as glycosyl donors and a wide array of promoter systems have been developed. Due to the selective activation conditions and excellent stability in the presence of non-thiophilic promoters, this type of donor has also found application as an acceptor.

Thioglycosides can be prepared in several different ways, but are most commonly synthesized by reacting the thiol with an anomeric acetate in the presence of a Lewis acid. Their stability provides a long shelf-life and makes them suited for protecting group manipulations. Furthermore, thioglycosides can within two steps be converted into the most common glycosyl donors, as shown in Scheme 6.[74]



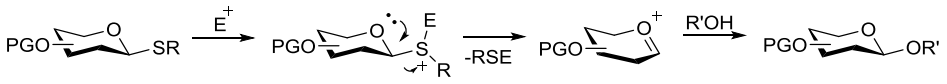
Scheme 6 Formation of thioglycosides and transformation hereof.

Trichloroacetimidate donors can be prepared through hydrolysis of the thioglycosides, followed by the general conditions for formation of imidate donors. Glycosyl bromides can be formed in one step by titration with bromine and glycosyl fluorides can be formed directly by employing (diethylamino) sulfur trifluoride (DAST).

The above mentioned properties make thioglycosides convenient in the assembly of oligosaccharides.[75] Thioglycosides can be activated by various electrophiles, as shown in Scheme 7, but remain inert under other

glycosylation conditions.[76] These electrophiles include a range of heavy metal salts and thiophilic reagents, such as mercury(II) salts[77] ($HgSO_4$, $Hg(OAc)_2$ and $HgCl_2$), MeOTf[78–80], DMTST[81,82], MeSOTf[83,84], IDCP[85–87], IBr[88,89] and NIS-TfOH[90–92].

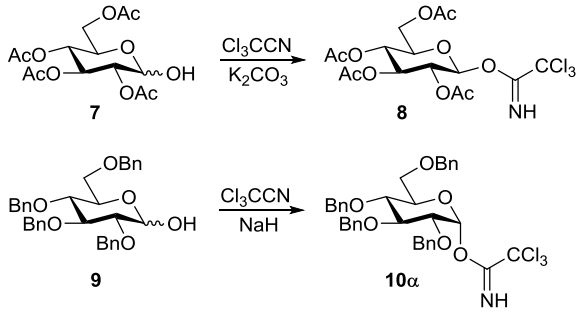
Soft electrophiles can be used to activate thioglycosides by forming a sulfonium ion. The sulfonium ion is an excellent leaving group, which departs readily with assistance of the ring oxygen or neighboring group participation leading to the oxocarbenium ion or the acetoxonium ion, which can then react with the alcohol forming the glycosidic linkage.



Scheme 7 Activation of thioglycosides with electrophilic reagent.

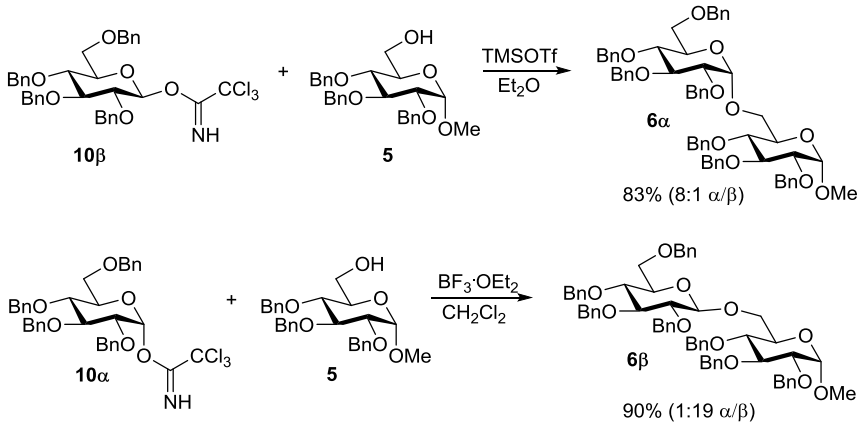
1.2.3. Glycosyl Trichloroacetimidates

Glycosyl acetimidates were first described by Pougny and Sinaÿ.[93] They achieved the formation 1,2-*cis* glycosidic linkages in good yields using this glycosyl donor.[94] The use of glycosyl acetimidates was further developed by Schmidt and Michel[95] introducing the trichloroacetimidate as a leaving group. Trichloroacetimidates are formed by reacting trichloroacetonitrile with the free hydroxyl group under basic conditions. Depending on the strength of the base either the kinetic β-trichloroacetimidate **8** or the thermodynamic α-trichloroacetimidate **10α** is formed, which is shown in Scheme 8.[96]



Scheme 8 Formation of α- and β-trichloroacetimidates.

Trichloroacetimidates are relatively stable under neutral and alkaline conditions. Under acidic conditions the trichloroacetimidate moiety is an excellent leaving group, and is commonly is activated with a Brønsted acid or a Lewis acid, e.g. $BF_3{\cdot}OEt_2$[95] or trimethylsilyl trifluoromethanesulfonate (TMSOTf)[97,98]. This method can be used to generate both 1,2-*cis*[99] and 1,2-*trans*[95] glycosides from the glycosyl trichloroacetimidate with the opposite anomeric configuration, as shown in Scheme 9.

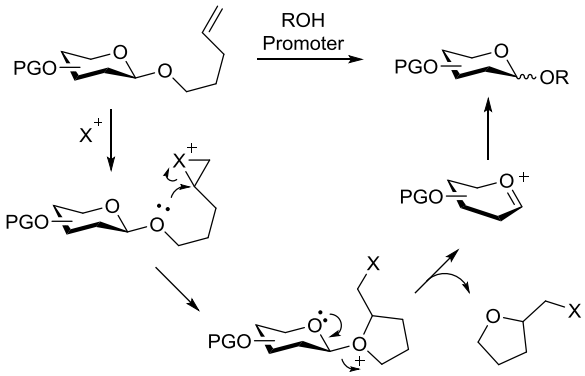


Scheme 9 Stereoselective glycosylation with glycosyl trichloroacetimidates.

1.2.4. *n*-Pentenyl glycosides

n-Pentenyl glycosides are a class of glycosyl donors that exhibit excellent stability under a range of conditions and can undergo common protecting

group manipulations without degradation. This class of glycosyl donors were first introduced by Fraser-Reid and co-workers.[100,101] The activation of *n*-pentenyl glycosides is rather sophisticated and occurs through halogenation of the double bond forming a cyclic aglycon, that ultimately acts as the leaving group, as shown in Scheme 10.



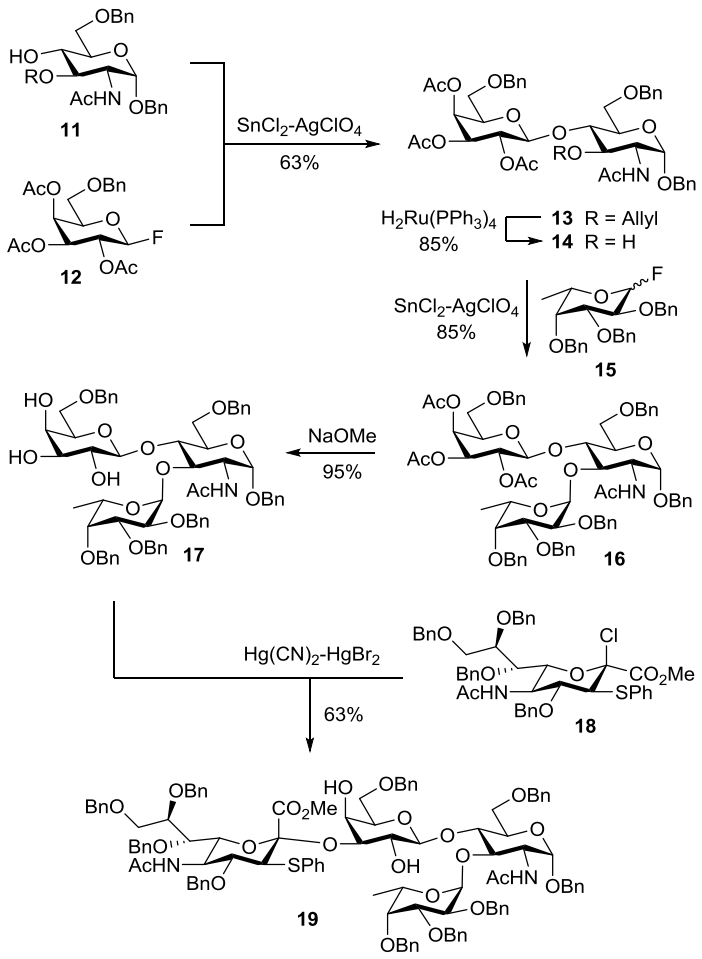
Scheme 10 Activation of *n*-pentenyl glycoside through halogenation of the terminal alkene.

Initially, *N*-bromosuccinimide (NBS) was used as the promoter for this type of glycosyl donor. Later, iodonium dicollidine perchlorate (IDCP) and *N*-iodosuccinimide (NIS) in combination with catalytic amounts of protic or Lewis acids were studied.[102] *n*-Pentenyl glycosides can be hydrolyzed and converted into glycosyl bromides in the same manner as thioglycosides.

Like thioglycosides, the properties of *n*-pentenyl glycosides make them useful in oligosaccharide assembly. Fraser-Reid and co-workers[103] also made observations regarding the differences in reactivity between *n*-pentenyl glycosides depending on the type of protecting groups employed. Further studies of this phenomenon led to the armed/disarmed strategy which will be discussed in section 1.3.3.

1.3 Strategies in Oligosaccharide Assembly

Lewis^X type fragments and similar structures have been used to showcase the different strategies and methods in the chemical synthesis of carbohydrate over the years. In Scheme 11 the stepwise approach for synthesis of oligosaccharides is illustrated by the synthesis of the sialyl Lewis^X fragment **19**, where the sugar chain is extended by one monosaccharide at the time.[104] The Lewis^X fragment **19** in Scheme 11 was synthesized by Nicolaou *et al.*[104] in 1991.



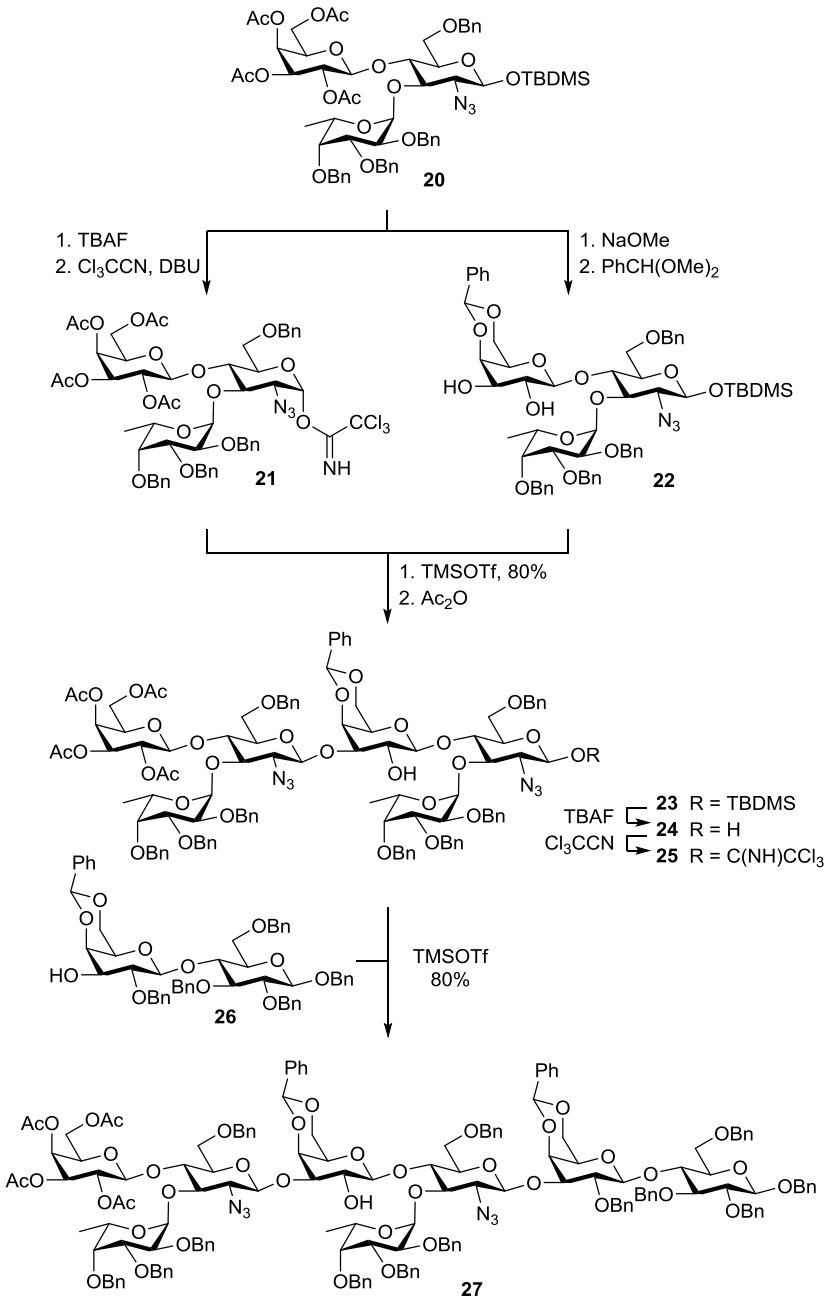
Scheme 11 Stepwise synthesis of tetrasaccharide **19** (Sialyl Le^x).

The tetrasaccharide fragment **19** then underwent reductive desulfurization and deprotection of the benzyl groups, and as a result the deprotected fragment was synthesized in 8 steps from the monosaccharide building blocks in 20% overall yield. These 8 steps included two deprotection steps of temporary protecting groups, which both proceeded in excellent yield. Noteworthy is the selective glycosylation of the trisaccharide **17**, which is exploiting the increased reactivity of the 3-position in galactose. The monosaccharide building blocks **11**, **12**, **15** and **18** were synthesized in 4 to 5 steps from phenyl 1-thio-β-D-galactose, the methyl ester of sialic acid, *N*-acetylglucosamine and L-fucose. The galactose, glucosamine and sialic acid building blocks **11**, **12** and **18** were synthesized in less than 40% each, thus showing the difficulties of forming building blocks efficiently and underlining the importance of continuing improvement of coupling conditions.

A convergent strategy in oligosaccharide assembly is most commonly preferred, since the yield often decreases rapidly for each glycosylation step in a stepwise synthesis, which is therefore often only desirable when coupling up to 4 monosaccharides. An example of an impressive convergent approach was showcased by Toepfer and Schmidt[105,106] in the synthesis of dimeric Lewis^x **27** (Scheme 12). The synthesis was also used to demonstrate the utility of trichloroacetimide donors developed by Schmidt and Michel[95] as previously discussed. Disaccharide building block **26** and trisaccharide building block **20** were also synthesized employing this type of donor. The trisaccharide building block **20** was synthesized from the monosaccharide building blocks in 40% yield. Both the coupling between the two trisaccharides **21** and **22**, along with the coupling between the hexasaccharide **25** and the disaccharide building block **26**, proceeded in 80% yield.

The use of more stable glycosyl donors with specific activation conditions have opened up for further improvements in the block wise synthesis. These improvements include a range of different strategies and methods (*vide infra*). Despite useful developments, assembly of oligosaccharides

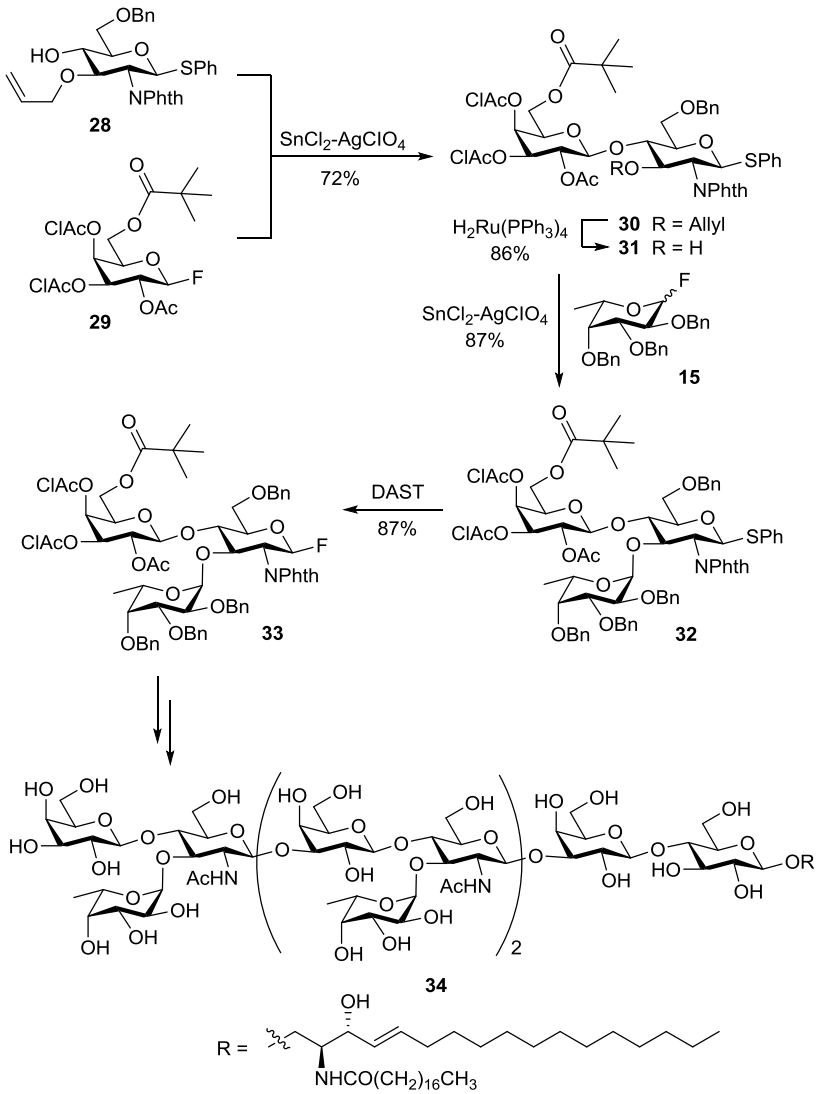
still requires a multitude of individual considerations to be made.[107] The most frequently used strategies are discussed below.



Scheme 12 Block synthesis of dimeric Lewis^x **27.**

1.3.1. Two-Stage Activation

Nicolaou and co-workers[108] early on realized the limitations relating to a linear synthesis of oligosaccharides, and therefore they introduced the two stage activation in 1984.



Scheme 13 Two stage activation employing glycosyl fluorides as donors and thioglycosides as acceptors.

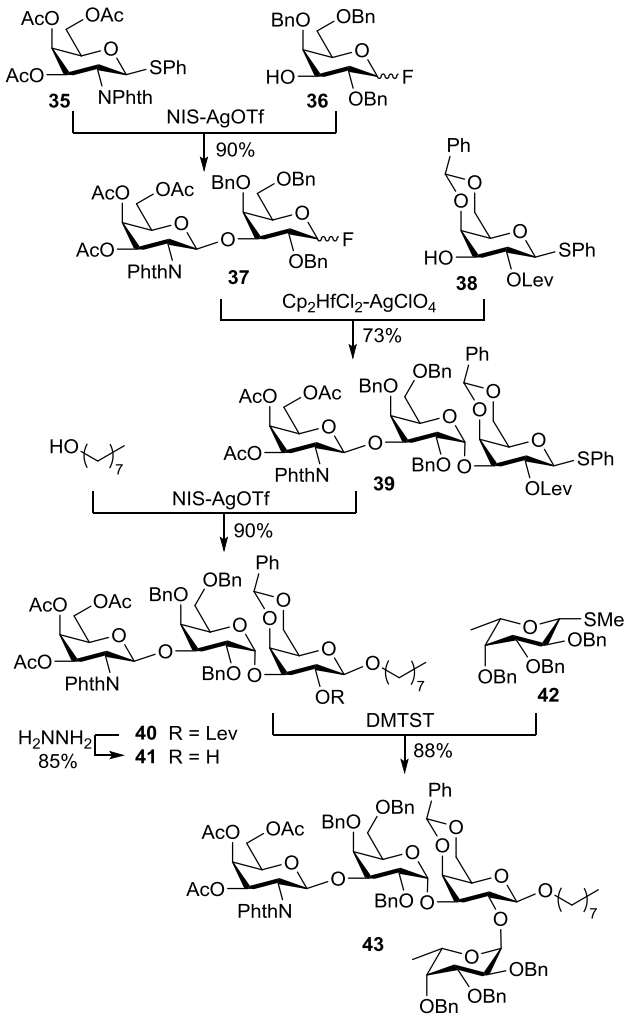
The strategy employs two different anomeric substituents A and B, where A plays the role of the glycosyl donor and B is stable under the given glycosylation conditions. Following the glycosylation reaction B is transformed into A.

Nicolaou and co-workers[108] first utilized this strategy by employing thioglycosides as acceptors, which were glycosylated with glycosyl fluorides. After the glycosylation, the thioglycosides were converted into glycosyl fluorides using DAST and NBS, and then used as the donor in the following glycosylation. This method was shown in the synthesis of a pentasaccharide belonging to the rhynchosporoside family.[109] Later Nicolaou and co-workers[110–112] showed this strategy in several papers synthesizing the Lewis^x oligosaccharides. The strategy is showcased in Scheme 13, where a trisaccharide building block **32** was assembled and subsequently used to synthesize a dodecasaccharide **34**.

1.3.2. Orthogonal Activation

Ogawa and co-workers[113] introduced the orthogonal glycosylation strategy in which two different types of glycosyl donors are employed. Both types of glycosyl donors should be inert towards the activation conditions of the other glycosyl donor, and thus both are able to function as acceptors. This strategy became possible with the development of stable donors, such as thioglycosides, *n*-pentenyl glycosides and glycosyl fluorides. By alternating the activation conditions an iterative process becomes possible. Ogawa and co-workers demonstrated the validity of this strategy with the synthesis of different oligosaccharides in the tetramer to heptamer range using thioglycosides and glycosyl fluorides. An example of the application of the orthogonal strategy is given in Scheme 14, where Ogawa and co-workers[114] synthesized a blood group B determinat **43** in this manner by employing glycosyl fluorides and thioglycosides as in the original paper. All steps from the protected monosaccharides **35**, **36**, **38** and **42** proceed in good yield underlining the orthogonality of both glycosyl donors towards the activation conditions of the other donor. The tetrasaccharide

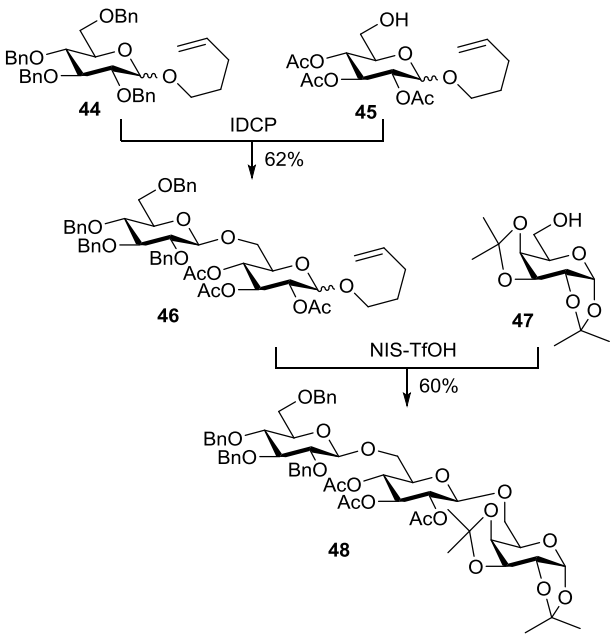
43 further undergoes deprotection of the hydroxyl groups and selective acetylation of the amine in excellent yields, which affords the unprotected tetrasaccharide in an overall 38% yield from the protected monosaccharide building blocks.



Scheme 14 Orthogonal synthesis of blood group B determinant made possible by applying glycosyl donors inert towards the others activation conditions of each other.

1.3.3. Armed/Disarmed Strategy

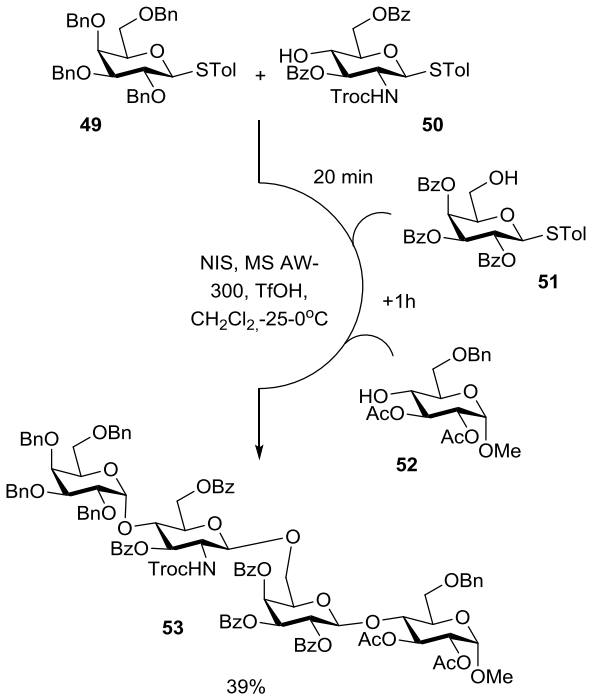
The armed/disarmed strategy is one of the most significant contributions in oligosaccharide assembly. It is based on observations by Fraser-Reid and co-workers on the reactivity of *n*-pentenyl glycosyl donors.[91,92,115] They discovered that *n*-pentenyl glycosides carrying electron donating protecting groups (e.g. benzyl groups) could selectively be activated over the electron withdrawing acetylated counterpart. Consequently, enough difference in reactivity was created by the use of different protecting groups to render the less reactive *n*-pentenyl donor almost inert with careful application of appropriate glycosylation conditions as shown in Scheme 15. The benzyl protected *n*-pentenyl glucopyranosyl donor **44** is activated by employing iodonium dicollidine perchlorate (IDCP), followed by activation of the acetylated *n*-pentenyl glucoside **45** with *N*-iodosuccinimide (NIS) and triflic acid.



Scheme 15 The armed/disarmed strategy. Chemoselective activation of glycosyl donors of the same type based on the difference in reactivity created by application of different protecting groups.

The observations leading to the armed/disarmed strategy have provided a tool for chemoselective differentiation between glycosyl donors of the same type. The arming or disarming effect of protecting groups have since then been explored with different glycosyl donors in many strategies.[116] One-pot glycosylation by using the armed/disarmed strategy is one of the most interesting strategies, where the most reactive donor is acting as such and the less reactive glycosyl donor is employed as the acceptor.

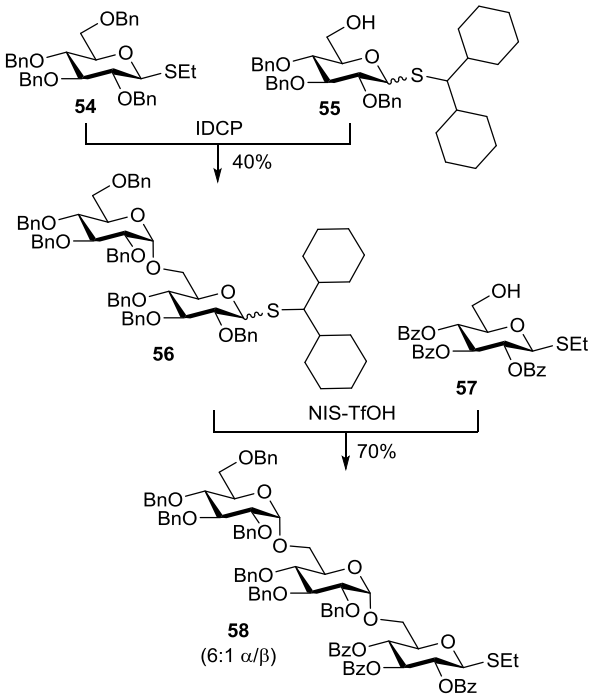
Wong and co-workers[117] established relative rate values based on competition experiments between tolyl thioglycosides with a range of different protecting group patterns. This knowledge was used to synthesize several different saccharides, also including one-pot glycosylations. Synthesis of the tetrasaccharide **53** done by Wong and co-workers[117] is showcased in Scheme 16.



Scheme 16 One-pot glycosylation employing the armed/disarmed strategy.

The first acceptor **50** to be glycosylated is approximately 100 times less reactive than the donor **49** and the following acceptor **51** is approximately 1300 times less reactive than the initial donor **50**. The final tetrasaccharide **53** was obtained after 3 couplings in overall 39% yield.

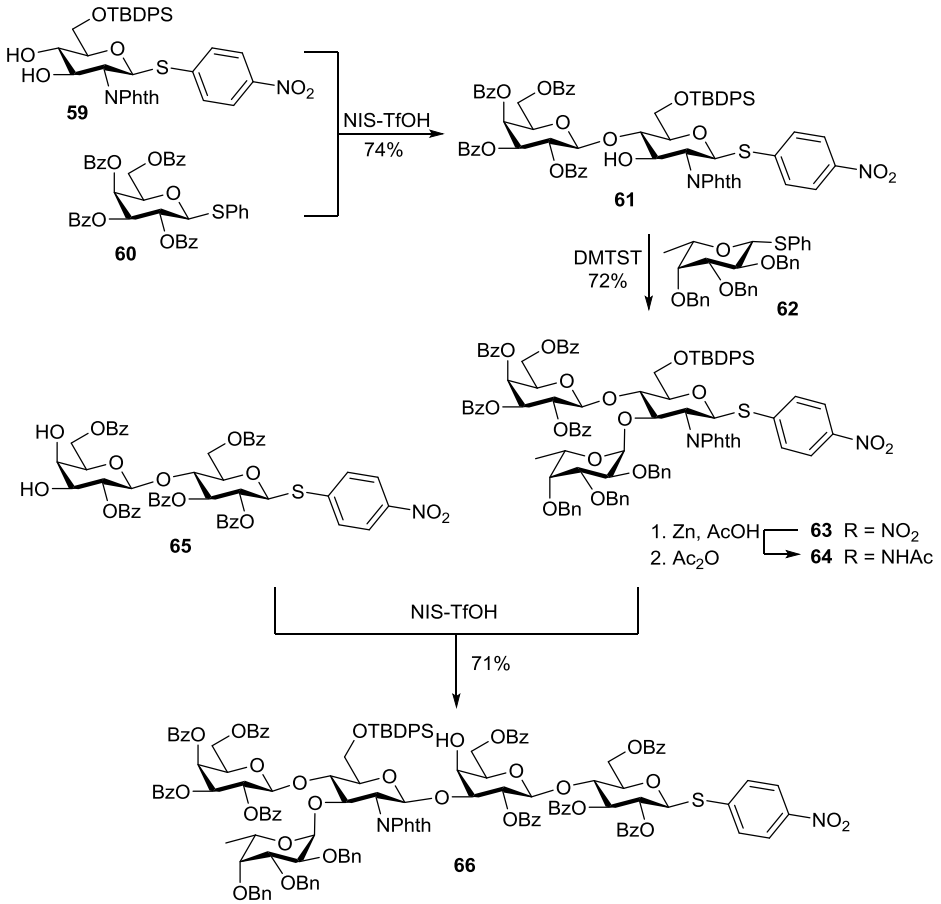
The armed/disarmed approach has since the observations by Fraser-Reid and co-workers been expanded to reactivity differences based on torsional differences[118] and the bulkiness of thioglycoside aglycons.[119] Boons and co-workers[120] employed thioglycosides and combined influence of the protecting groups with different types of aglycons as shown in Scheme 17. Dicyclomethyl thioglucoside **55** was glycosylated chemoselectively with ethyl thioglucoside **54**, and both being benzyl protected shows that sheer bulk can create the reactivity difference necessary for selective activation. α-Linked product **56** was isolated in 40% yield and was selectively activated over the disarmed ethyl thioglucoside **57** in the following glycosylation affording the trisaccharide **58** in 70% yield as a 6:1 α/β mixture.



Scheme 17 Application of bulky algylons creating differences in reactivity.

1.3.4. Active/Latent Strategy

The active/latent strategy is in line with the two-stage activation, although the strategy is now based on one type of donor with modifications to the aglycon, which creates the possibility for chemoselective activation. This concept was demonstrated by Cao *et al.*[121] in the synthesis of the Lewis^X fragment **66** by employing *p*-nitrophenyl thioglycoside **59** as a latent donor, which was subsequently converted into the active donor **64** (Scheme 18).



Scheme 18 Active-latent strategy applied to synthesis of Lewis^X fragment employing different by activated thioglycosides as donors and the deactivated *p*-nitrophenyl thioglycoside as acceptor.

In the first two couplings the phenyl thioglycosides **60** and **62** were employed as donors and the *p*-nitrophenyl thioglycoside acceptor remains inert. Hereafter, the latent trisaccharide **63** was converted into the active donor **64** and coupled to the latent disaccharide **65**. The conversion from latent to active donor was achieved in 84% yield.

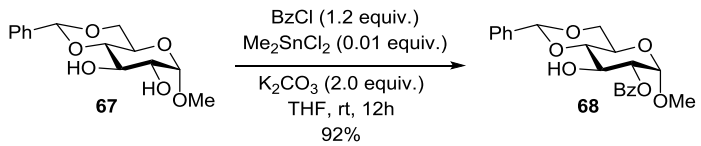
1.4 Regioselective Manipulations of Sugars

The field of synthetic carbohydrate chemistry is dominated by protecting group chemistry. Zhu and Schmidt[4] recently published a review on the developments for forming of the *O*-glycosidic linkage in which was stated "Glycosylation of a suitable unprotected acceptor, which generally contains only one free hydroxyl group,...". This statement is a testament to the necessity of protecting groups in carbohydrate chemistry, especially considering the fact that 3 different monosaccharides can be combined into trisaccharides in 1056 ways.[122] As described in the previous section, elaborate strategies for lending regio- and stereoselectivity have been developed over the years. Protecting groups are an essential part of this process by preventing unwanted side reactions, tuning reactivity and neighboring group participation. However this is adding protection and deprotection steps for each type of protection group, thus making the synthesis significantly longer. Many efforts have been made to shorten sequences in the synthesis of carbohydrates by reducing the number of protecting groups involved. One such effort is the regioselective manipulation of partial and fully unprotected carbohydrates.

A key factor for regioselective manipulation of unprotected carbohydrates is the distinction between the hydroxyl groups. Generally, primary alcohols can undergo selective manipulation over secondary hydroxyl groups, which is elegantly demonstrated by Porter *et al.* in the selective tritylation protocol of the primary alcohol.[123] In this section, the focus is mainly on regioselective metal-mediated manipulation of unprotected or partially protected glycopyranosides.

The slight difference in the inherent reactivity between the secondary hydroxyl groups have been exploited in the selective placement of protecting groups for decades, most notably by employing tin reagents for regioselective acylation, alkylation, phosphorylation and sulfonylation, but also glycosylation which will be dealt with in section 1.4.1.[124,125] Organotin reagents have also been applied in mild regioselective oxidation of sugars to ketosugars.[126]

Commonly, organotin reagents have been applied in stoichiometric amounts, although more recent publications have investigated the application of organotin reagents in catalytic to sub-stoichiometric quantities. Matsumura an co-workers[127,128] employed catalytic dimethyltin(IV) chloride for selective benzoylation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside **67** in 92% yield, demonstrating a clear preference for the hydroxyl group 1,2-*cis* to the methyl ether as shown in Scheme 19.



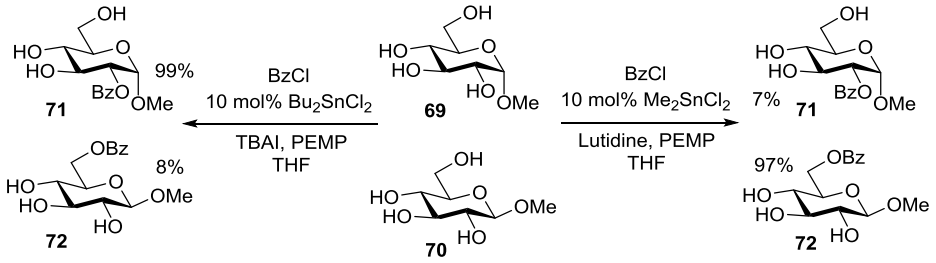
Scheme 19 Selective monobenzoylation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside **67** by employing dimethyltin dichloride.

Selective sulfonylation of monosaccharide substrates by employing catalytic quantities of organotin reagent was described around the same time.[129–131]

In 2008 Onomura *et al.*[132] published a systematic study of the regioselective benzoylation of fully unprotected pyranosides with catalytic dimethyltin dichloride. An interesting order of benzoylation emerged by demonstrating a superior selectivity for the equatorial position in *cis*-vicinal hydroxyl groups. In the absence of *cis*-vicinal hydroxyl groups, the equatorial hydroxyl groups *cis*-vicinal to a methyl ether was preferred. Lastly, in the absence of any 1,2-*cis* vicinal hydroxyl groups, the inherent most reactive hydroxyl group or the least hindered position was benzoylated, which accounts for benzoylation of the primary hydroxyl groups in methyl β-D-glucopyranoside and the 4-position in methyl β-D-xylopyranoside.

Muramatsu and Takemoto[133] utilized the molecular recognition ability of catalytic organotin reagents to facilitate regioselective functionalization of glucose substrates in Scheme 20. This functionalization was done to make the separation of the anomers less demanding. Dibutyltin dichloride favored benzoylation of *cis*-vicinal hydroxyl groups in methyl α-D-

glucopyranoside **69** rather than reaction with methyl β-D-glucopyranoside **70**, whereas dimethyltin dichloride favored benzoylation of the primary hydroxyl group in methyl β-D-glucopyranoside **70**.



Scheme 20 Selective benzoylation depending on organotin reagent.

The catalytic organotin mediated acylation of pyranosides was extended to alkylation, when dibutyltin oxide was recently employed in regioselective benzylations. The regioselective benzylation was done successfully under solvent-free conditions[134] and in DMF/MeCN[135] under otherwise similar conditions.

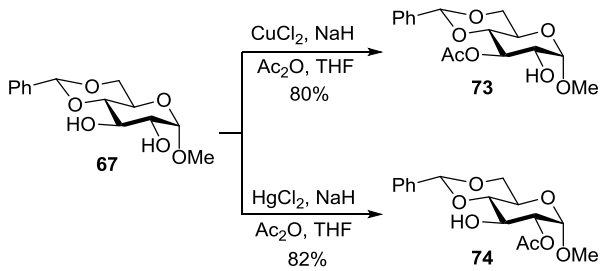
Stannylene acetals can be formed with 1,2-*cis* diols in pyranosides and alkylation of these predominantly result in equatorial ethers, which for stannylene acetals of galactopyranosides will lead to alkylation of the 3-position.[136,137] Veyriéres *et al.*[138] early on made the observation that 5 membered stannylene acetals with 1,2-*cis* diols were preferred to the formation of 6-membered acetal rings with the primary hydroxyl group in galactopyranosides. These observations regarding the need for *cis*-vicinal hydroxyl groups were in agreement with previous findings in nucleotides[139] and carbohydrates.[136,137,140] Selective functionalization of vicinal hydroxyl groups was later developed by employing catalytic amounts of dialkylorganotin(IV) reagents to form stannylene acetals.[128,130,132,141]

The advances in the application of organoboron reagents are much in line with the history of the tin-mediated manipulations and will be discussed in depth in section 1.4.2. In brief, Aoyama and co-workers[142,143] reported the use of boronic acid reagents in regioselective alkylation of sugars and continued onto conducting glycosylation in the same manner. Taylor and co-workers[144] extended the application of organoboron reagents in

manipulation of sugars to catalytic quantities of a borinic acid. Benzoylation with organoboron reagents of secondary hydroxyl groups could, contrary to organotin reagents, not be achieved in the presence of primary hydroxyl groups.[144] Their work extends past regioselective acylation, and alkylation[145] and sulfonylation[146] to glycosylation, which will be discussed later.

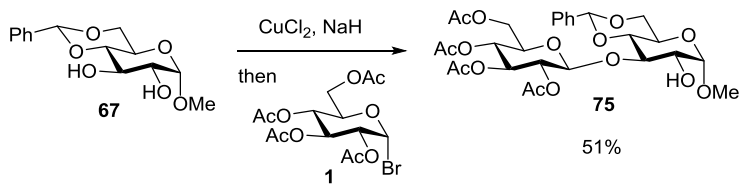
Transition metals have been explored in regioselective manipulation to a much smaller extend than tin and boron reagents, although it is known that some transition metals can form complexes with carbohydrates.[147,148] In recent years, more attention has been given to transition metal-mediated regioselective transformations. Copper(II), mercury(II), nickel(II), silver(I) and molybdenum have been investigated, as well as the more expensive metals iridium and palladium.

In 1982, Eby and Schuerch[149] described regioselective alkylation of gluco-, galacto- and mannopyranosides employing stoichiometric copper(II) chloride and sodium hydride. Both benzylation and allylation of the methyl 4,6-*O*-benzylidene-α-D-pyranoside derivatives lead to reaction with the 3-position for all cases. Alkylation was also performed on pyranosides with free hydroxyl groups in the 4 and 6 position, which interestingly lead to reaction with the 4-position with the alkyl halide. Schuerch *et al.*[150] expanded investigations to encompass acylation by employing both copper(II) and mercury(II) salts. In methyl 4,6-*O*-benzylidene-α-D-pyranoside **(67)** copper(II) chloride directed acylation towards the 3-position and mercury(II) chloride displayed the opposite preference directing acylation towards the 2-position as shown in Scheme 21.



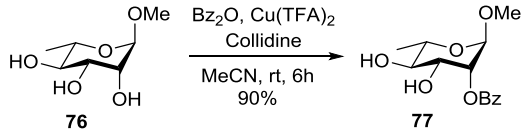
Scheme 21 Regioselective acetylation employing copper(II) and mercury(II) salts.

Suthers and co-workers[151] continued in the footsteps of Schuerch and co-workers[149] almost two decades later by employing copper(II) chloride for acylation of ethyl 4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside. The acylations employ benzoyl, pivaloyl and acetyl chloride and moderate to good yields are achieved for reaction with the 3-position. Suthers *et al.*[152] applied similar conditions to glycosylation of methyl 4,6-*O*-benzylidene α-D-glucopyranoside **67** with acetobromoglucose **1** resulting in 51% yield shown in Scheme 22.



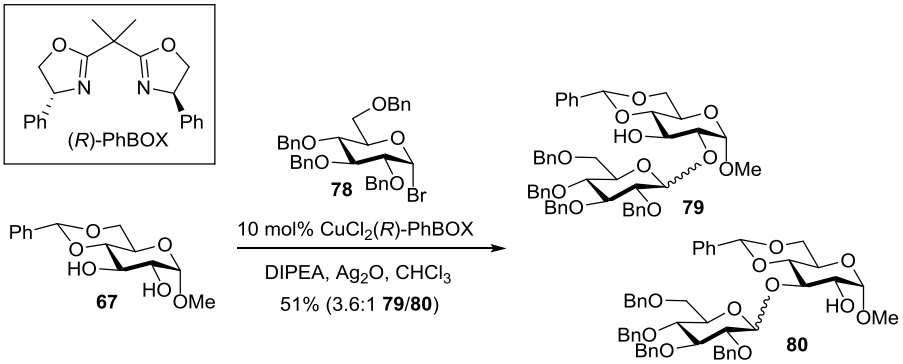
Scheme 22 Regioselective glycosylation employing copper(II) chloride.

Another decade later, Evtushenko studied the complex formation of transition metals with carbohydrates, and in this way affected regioselective benzoylation of glucopyranosides with copper reagents as directing agent. Using methyl α-L-rhamnopyranoside **(76)** as substrate, Evtushenko[153] demonstrated regioselective benzoylation of the 2-position with copper(II) trifluoroacetate, copper(II) perchlorate and copper(II) triflate in the presence of collidine and with little influence of the solvents as shown in Scheme 23. The copper reagent was suggested to form complexes with 1,2-*cis* vicinal hydroxyl groups or hydroxyl groups *cis*-vicinal to an alkoxide. Otherwise, in the absence of 1,2-*cis* vicinal hydroxyl groups, Evtushenko found the coupling to occur with the hydroxyl group possessing the strongest inherent selectivity, such as the primary alcohol. It was speculated that chelation with the copper(II) ion would lead to deprotonation of one hydroxyl group by the base. The increased ionization of this hydroxyl group raised the inherent nucleophilicity enabling reaction with benzoyl anhydride.[153]



Scheme 23 Regioselective benzoylation of compound **76** with $Cu(TFA)_2$.

At the same time, Allen and Miller[154] were investigating regioselective manipulation of methyl 4,6-*O*-benzylidene-D-pyranoside derivatives by employing copper(II) salts with chiral ligands. In one case they glycosylated methyl 4,6-*O*-benzylidene-α-D-glucopyranoside **(67)** with 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide **(78)** achieving regioselectivity at the 2-position in the ratio of 3.6:1 over the 3-position as shown in Scheme 24.



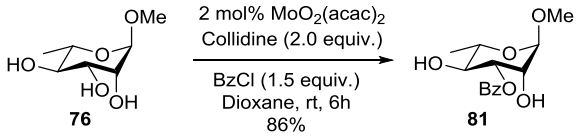
Scheme 24 Regioselective glycosylation employing chiral copper reagent.

Dong and co-workers[155] performed regioselective manipulation with chiral copper reagents on substrates, which were only protected at the primary hydroxyl groups and achieving acylation of equatorial hydroxyls groups *cis*-vicinal to either a hydroxyl or an alkoxide group.

Gangadharmath and Demchenko[156] investigated nickel(II) chloride mediated manipulations of vicinal equatorial hydroxyl groups in methyl 4,6-*O*-benzylidene-α-D-glucopyranoside and -galactopyranoside. In the presence of 1,2-vicinal axial alkoxide groups, i.e. α-anomer, regioselectivity was observed for both alkylation and acylation. No regioselectivity was observed in methyl 4,6-*O*-benzylidene-β-D-glucopyranoside.

Silver(I) oxide was investigated by Ye *et al.*[157] for the regioselective influence in tosylation, acetylation and benzoylation with 4,6-*O*-benzylidene protected pyranosides. Regioselective functionalization was achieved in good yields, however selectivity was highly dependent on substrate and the protecting groups implemented.

Evtushenko[158,159] conducted protecting group manipulations by employing molybdenum complexes in catalytic quantities. This work demonstrated an excellent ability of $MoO_2(acac)_2$ and $MoCl_5$ for regioselective acylation of pyranosides with 1,2-*cis* vicinal hydroxyl groups in the absence of primary hydroxyl groups. The system was optimized on methyl α-L-rhamnopyranoside **(76)** and illustrated for benzoylation in Scheme 25.

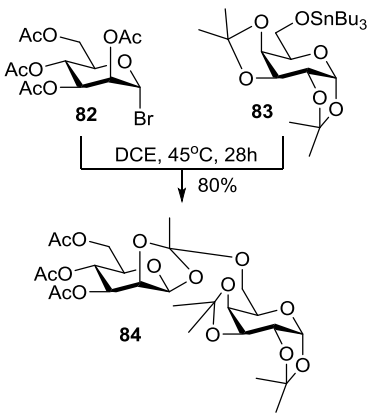


Scheme 25 Regioselective benzoylation with catalytic $MoO_2(acac)_2$.

1.4.1. Tin-Mediated Glycosylation

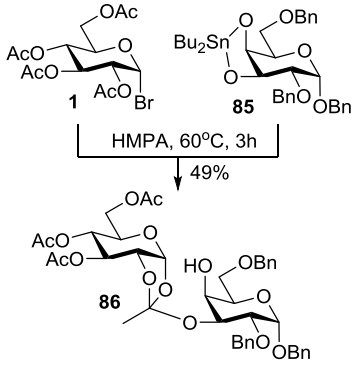
In this section, previous publications regarding regioselective glycosylation involving organotin(IV) reagents are considered. Organotin(IV) reagents have been employed extensively in regioselective transformations of sugars. As described earlier, manipulations with organotin(IV) reagents proceed through two different tin-species, either trialkyltin ethers or stannylene acetals.[124] Formation of trialkyltin ethers and stannylene acetals raises nucleophilicity of the hydroxyl groups, which was exploited by Hanessian *et al.*[125] in regioselective acylation and alkylation of carbohydrates.

Ogawa and Matsu[35] achieved the first glycosylation by applying organotin(IV) reagents and simple alcohols in 1976. They employed trialkyltin reagents to enhance nucleophilicity of hydroxyl groups belonging to both primary and secondary alcohols, followed by glycosylating with acetobromomannose **82** resulting in the orthoester product **84** shown in Scheme 26.



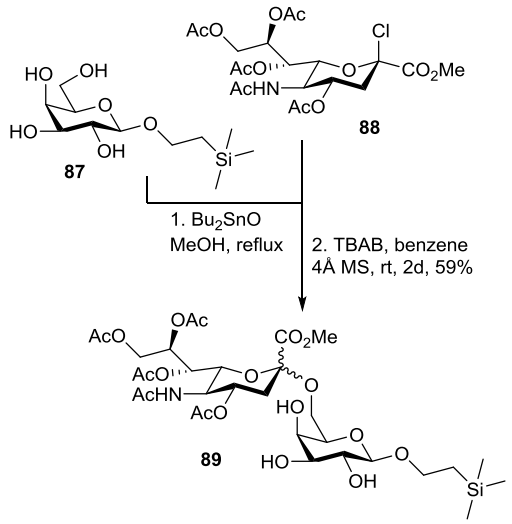
Scheme 26 Glycosylation employing trialkyltin by Ogawa and Matsu.

Shortly after Ogawa and co-workers application of trialkyltin, Augé and Veyiéres[160] employed dialkylstannylene acetals, such as compound **85**, in glycosylation with acetobromoglucose **1** achieving the 3-linked orthoester product **86** as shown in Scheme 27.



Scheme 27 Application of stannylene acetal in glycosylation.

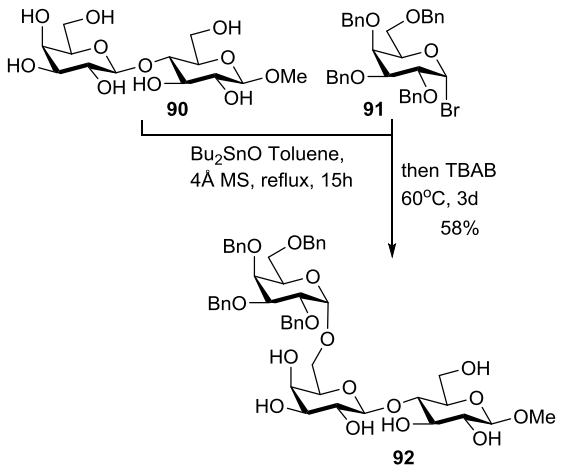
Years later, Hasegawa *et al.*[161] formed the stannylene acetal from fully unprotected 2-(trimethylsilyl)ethyl-β-D-galactopyranoside **(87)**, which was then utilized in glycosylation with methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-β-D-galacto 2-nonu-lopyranosyl chloride)onate **(88)**, resulting in an overall 59% yield of the 1,6-linked product **89** in an anomeric mixture as demonstrated in Scheme 28.



Scheme 28 Glycosylation of fully unprotected galactopyranoside through stannylene intermediate.

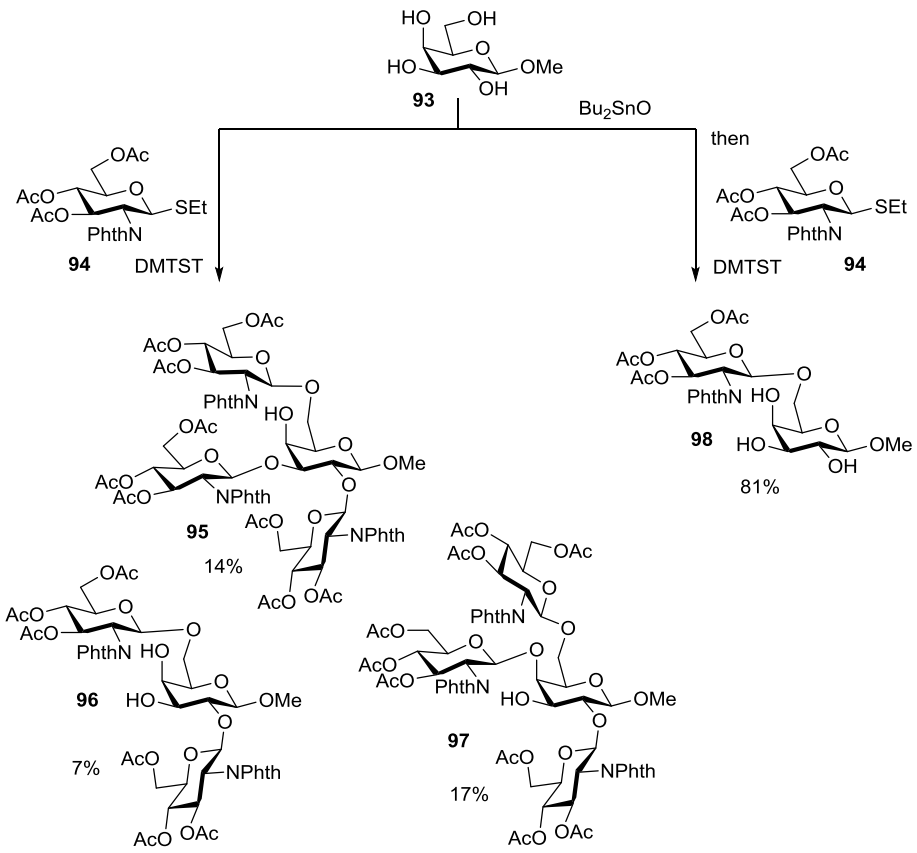
Martin-Lomas *et al.*[162] glycosylated methyl β-lactoside **(90)** with 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl bromide **(11)** in a similar manner.

Firstly, acceptor **90** was heated together with dibutyltin(IV) oxide to reflux in toluene, and then donor **91** and TBAB were added followed by stirring for 3 days, which resulted in the trisaccharide **92** in good yield as presented in Scheme 29.



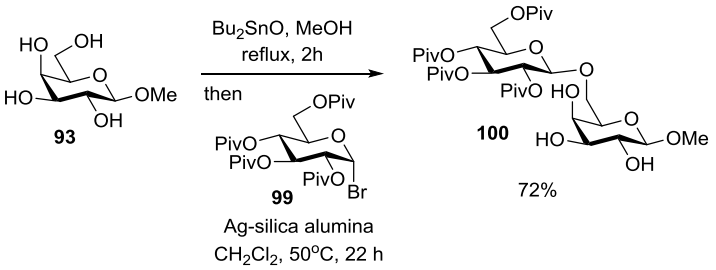
Scheme 29 Tin-mediated glycosylation of methyl β-lactoside **(90)** with 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl bromide **(91)** resulting in the trisaccharide **92**.

Oscarson *et al.*[163] employed stannylene acetals to achieve the formation of the 1,6-linkage with protected thioglycosides as donors and fully unprotected methyl β-D-galactopyranoside **(93)** as acceptor in moderate to good yields. Ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside **(94)** was employed as the donor in the glycosylation of the stannylene derivative of methyl β-D-galactopyranoside **(93)**, which resulted in the 1,6-linked product **98** in 81% yield. A control reaction without the tin reagent was conducted to establish the influence on the regioselectivity by dibutyltin(IV) oxide, which resulted in a mixture of tri- and tetrasaccharides **95-97** with no apparent selectivity as shown in Scheme 30.



Scheme 30 Similar reactions w/o dibutyltin(IV) oxide. Without organotin reagent a mixture of saccharides were obtained and with organotin reagent the 1,6-linked disaccharide **98** was obtained.

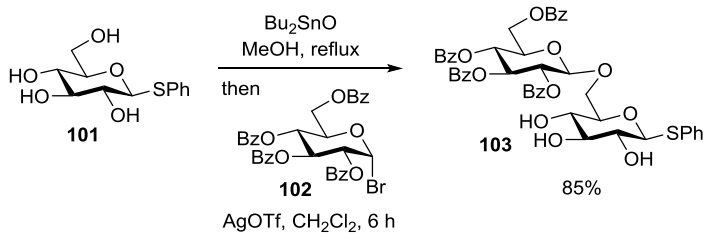
Kaji *et al.*[164] performed glycosylation of fully unprotected methyl β-D-galactopyranoside **(93)** by first forming the stannylene acetal species and then glycosylating it with the acyl protected glucopyranosyl bromide **99**, resulting in 72% of the 1,6-linked disaccharide product **100** as illustrated in Scheme 31.



Scheme 31 Regioselective glycosylation of methyl β-D-galactopyranoside **(93)** through the stannylene intermediate.

Kaji *et al.*[165] further investigated regioselective glycosylation of methyl β-D-galactopyranoside **(93)** in the same setup as in Scheme 31. By using tetrabutyl ammonium fluoride (TBAF), they managed to shift the regioselectivity to form the 1,3-linked disaccharide product in 42% yield instead.

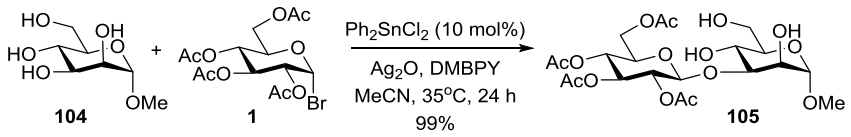
Maggi and Madsen[166] developed a protocol for stannylene-mediated regioselective glycosylation of unprotected thioglycopyranoside acceptors with disarmed glycopyranosyl bromides giving access to the 1,6-linked products. Employing phenyl 1-thio-β-D-glucopyranoside **(101)** as the acceptor and perbenzoylated glucopyranosyl bromide **102** as the donor formation of the 1,6-linked disaccharide product **103** was achieved in 85% yield as shown in Scheme 32.



Scheme 32 Stannylene-mediated regioselective glycosylation of thioglycoside acceptor.

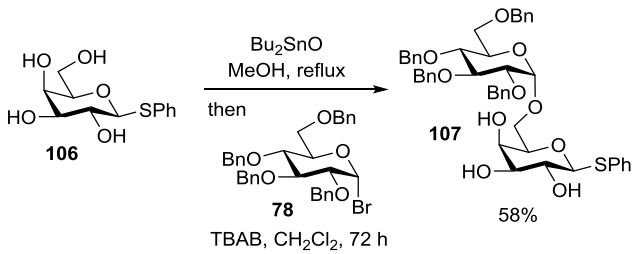
Muramatsu and Yoshimatsu[167] published work where they regioselectively glycosylated the 3-position of methyl α-D-mannopyranoside **(104)** with acetobromoglucose **1**. They achieved this by using catalytic amounts of an

organotin reagent with a pyridine type base and silver(I) oxide as the promotor in acetonitrile as shown in Scheme 33.



Scheme 33 Regioselective glycosylation of methyl α-D-mannopyranoside **(104)** employing catalytic amounts of organotin reagent.

Niedbal and Madsen[168] explored stannylene-mediated regioselective glycosylation by employing the reactive perbenzylated glycopyranosyl bromides together with tetrabutylammonium bromide (TBAB) for activation. Using this protocol as illustrated in Scheme 34, the formation of the 1,6-α-linked product **107** was achieved in 58% yield with phenyl 1-thio-β-D-galactopyranoside **(106)** as the acceptor and perbenzylated glucopyranosyl bromide **78** as the donor.

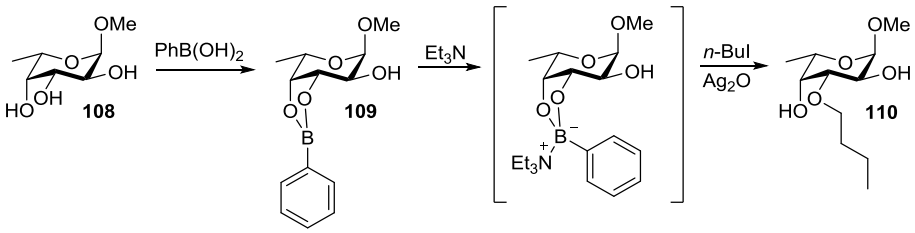


Scheme 34 Formation of α 1,6-linkage by regioselective glycosylation of thioglycoside.

1.4.2. Boron-Mediated Glycosylation

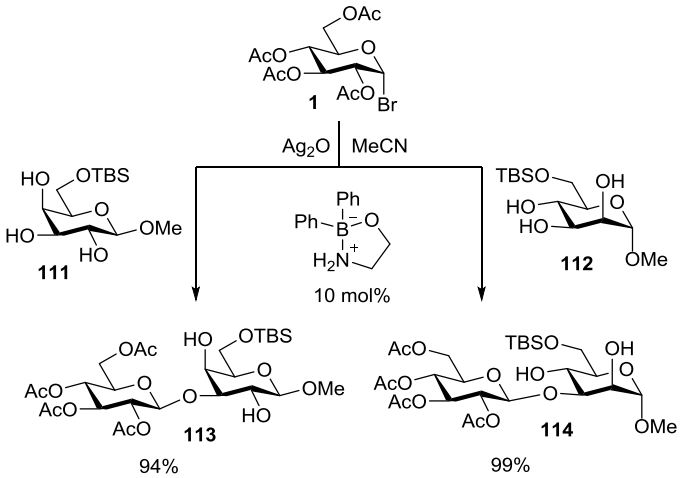
Boronic acid complexes of carbohydrates were first reported five decades ago by Ferrier and Prasad.[169,170] They employed boronic acids as protecting groups for diol functionalities in the 3-*O* alkylation of benzyl α-D-xylopyranoside. In more recent years, boronates have recieved much attention for activating hydroxyl functionalitites. Aoyama *et al.*[142] selectively alkylated the 3-position of the arylboronic acid complex of methyl α-L-fucopyranoside **(108)** as shown in Scheme 35 and continued

onto reporting the first regiospecific glycosylation with arylboronic acid derivatives in combination with a Lewis base in 1999.[143] Activation of the boron derivative was ascribed to interaction of the boron center with the Lewis base instead of masking the hydroxyl groups from the reaction. Regiospecificity was observed for 1,2-diols and 1,3-diols involving a primary hydroxyl group at the least hindered B-O moiety, hereby resulting in glycosylation of the boronate in the order of primary > secondary (equatorial) > secondary (axial). They continued to study selectivity toward the 3/6-position in glucoside, galactoside, mannoside, and fucoside while employing their boronic acid derivatives. Selectivity was dependent on whether the carbohydrates were partially protected.[171]



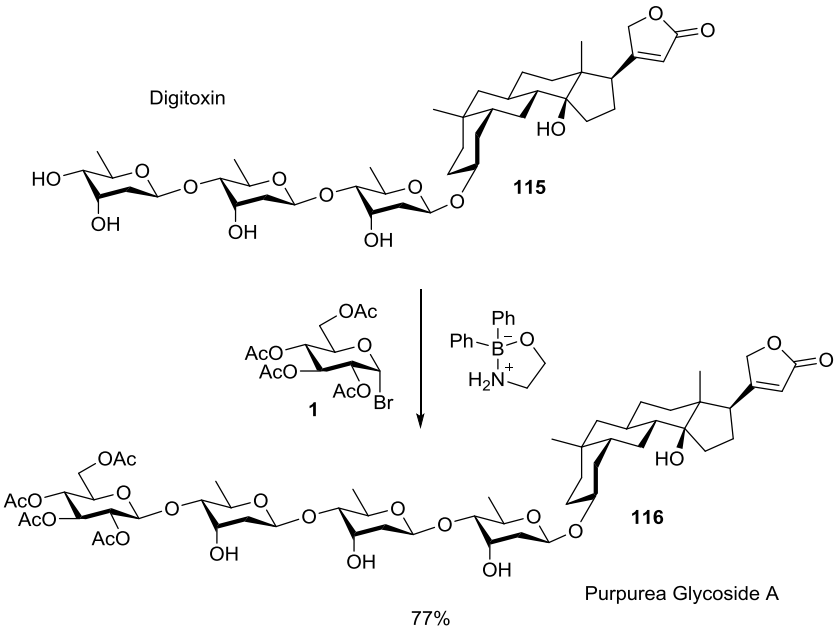
Scheme 35 Regioselective alkylation employing boronic acid.

Taylor and co-workers[144–146] employed diarylborinic acids to obtain increased nucleophilicity *via* the tetracoordinated diol complex without the use of a Lewis base, which was first explored in regioselective functionalization of methyl α-L-fucopyranoside. Diarylborininc acid was used to direct regioselective glycosylation of 6-*O* protected mannose and galactose derivatives under Koenigs-Knorr type conditions, affording the 1,3-linked products in good to excellent yields, which is shown for two cases of partial protected acceptors **111** and **112** in Scheme 36.[172]



Scheme 36 Regioselective glycosylation of acceptors employing borinic acid in catalytic amounts.

The protocol developed by Taylor and co-workers[173] was showcased in the regioselective glycosylation of Digitoxin **115** (Scheme 37) with aceto-bromoglucose **1**, which contains four unprotected hydroxyl groups.

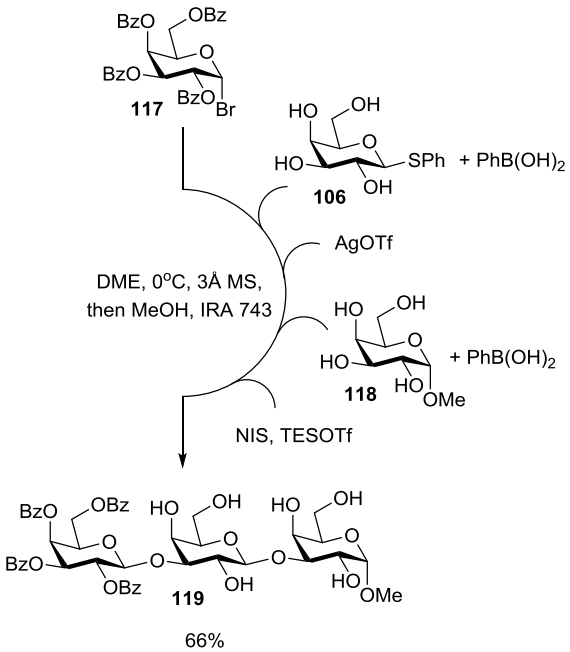


Scheme 37 Regioselective glycosylation of Digitoxin employing diarylborinic acid.

Application of the protocol with 25 mol% diarylborinic acid resulted in the 1,2-diol of Digitoxin **115** being glycosylated regioselectively under Koenigs-Knorr conditions and afforded protected tetrasaccharide Purpurea Glycoside A **116** in 77% yield.

Kaji and co-workes[174] demonstrated the ability of arylboronic acids to deactivate the 4,6-diol in β-D-galactopyranosides *in situ*, and in this way serves as a temporary protecting group, which caused the masked acceptor to form the 1,3-linked disaccharide with fully benzoylated thioglycoside donors in good yield (66%-78%).

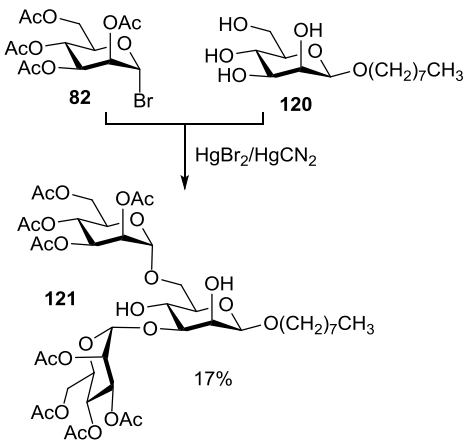
This type of *in situ* masking was further explored by Fenger and Madsen[175], where unprotected thioglycosides were employed as acceptors and glycosylated with benzoylated glycosyl bromides. The best results were achieved with phenyl 1-thio-β-D-galactopyranoside as the acceptor and benzoylated glucopyranosyl bromide as the donor. Furthermore, the trisaccharide **119** made up by 1,3-linked galactose units was synthesized in one-pot fashion using this protocol in an overall yield of 66%.



Scheme 38 Fenger *et al.*[175] employed boronic acids in one-pot glycosylation.

1.4.3. Random Glycosylation of Unprotected Acceptor

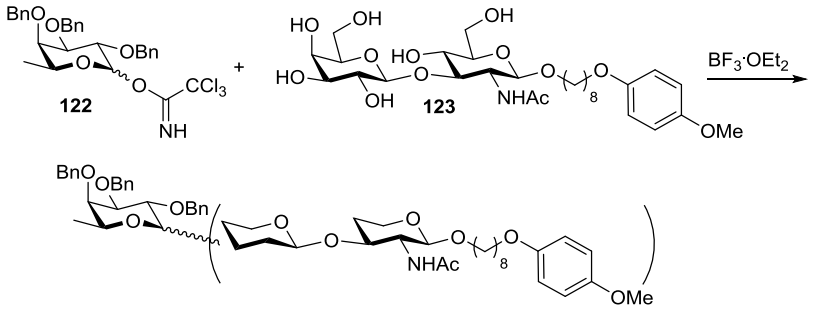
One other type of glycosylation of unprotected acceptors is the random glycosylation, which is only relying on the inherent selectivity of the sugar to determine the glycosylation product. This way of obtaining the glycosylation products is usually avoided, since many by-products are obtained, which results in low yield and making purification difficult.
Hindsgaul and co-workers[176] had a creative approach in securing their trisaccharide **121**. They employed the fully unprotected mannose acceptor **120**, which was glycosylated using 2.5 equivalents of acetobromomannose and $HgBr_2/HgCN_2$ as promoters, in order to afford octyl 3,6-di-*O*-(α-D-mannopyranosyl)-β-D-mannopyranoside **(121)** (Scheme 39). A mixture of mono-, di- and trisaccharides was obtained with no protecting group present to block other hydroxyl groups, and no reagent to direct the glycosylation of the acceptor. Contrary to the main product, the by-products contained vicinal diols, which were decomposed by subjecting the mixture to periodate oxidation and affording the desired acetyl protected trisaccharide in 17% yield.



Scheme 39 Random glycosylation to a trisaccharide employing unprotected β-D-mannopyranoside **120** as the acceptor.

This approach is not universal, since the substrates are simple and inherent selectivity is pronounced in mannose substrates, but it is another way of approaching regioselective glycosylation chemistry.

Hindsgaul and co-workers[177,178] later aimed to obtain libraries of trisaccharides for biological screening by the use of random glycosylation, in which unprotected carbohydrate acceptors were employed. All possible trisaccharides should ideally be obtained in equal amounts. Statically, each isomer should be obtained in 16-17% yield. The random glycosylation was initially done through fucosylation of unprotected disaccharides as shown in Scheme 40, which in case of acceptor **123** resulted in 12% of α(1,4), 22% of α(1,6), 19% of α(1,2'), 23% of α(1,3'), 8% of α(1,4') and 16% of α(1,6') using the tricloroacetimidate fucosyl donor **122**.



Scheme 40 Random fucosylation of unprotected disaccharide by Hindgaul and co-workers[177] resulting in 12% of α(1,4), 22% of α(1,6), 19% of α(1,2'), 23% of α(1,3'), 8% of α(1,4') and 16% of α(1,6').

This approach was later investigated for the preparation of a saponin library by Hui and co-workers[179] with rhamnose donors.

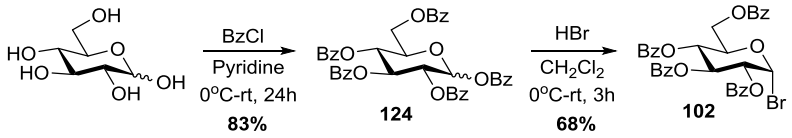
2 Investigation of Promoter Systems for Efficient Activation of Glycosyl Halides

In this chapter, promoter systems for efficient activation of disarmed glycosyl halides are investigated.[180] The Koenigs-Knorr reaction is employing glycosyl halides, and has been of paramount importance for more than a century, but efficient and benign promoter systems for activation of disarmed glycosyl halides are lacking. In the Koenigs-Knorr reaction and related protocols, glycosyl halides are generally activated by toxic and expensive metals salts as described previously. Instead, we wanted to investigate the use of inexpensive halogen electrophiles as promotors.

Several projects in our group have involved 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide **(102)**, since it is stable and easy to handle.[166,175,181] Due to the benzoyl protecting groups, this donor is highly disarmed and needs a powerful promoter for activation, hence making it a perfect choice for investigation of efficient non-metal promoter systems for unreactive glycosyl donors.

2.1 Initial screening

2,3,4,6-Tetra-*O*-benzoyl-α-D-glucopyranosyl bromide **(102)** was synthesized in two steps according to a literature procedure[181] from D-glucose mono-hydrate. Firstly, benzoyl protecting groups were introduced, and then the anomeric bromide was installed in an overall yield of 56%, as shown in Scheme 41.



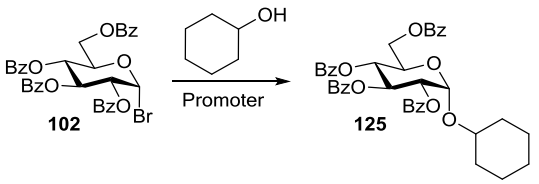
Scheme 41 Synthesis of 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide from D-glucose in two steps.

In the setup for initial screening of the promoter systems a simple acceptor was needed, since this would allow us to ascertain any potential activation capabilities. Sugars contain primarily secondary hydroxyl groups, and therefore cyclohexanol was chosen as a representation for a simple secondary alcohol and acceptor for the glycosylation with 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide **(102)**.

The non-metal promoter systems initially investigated were all based on halogen sources. These included iodine[44] and iodine monobromide[182], *N*-bromosuccinimide (NBS) and *N*-iodosuccinimide (NIS) known to promote activation of thioglycosides, and lastly zinc halides[36] were considered based on literature.

The setup for the initial screening of promoters is shown in Table 1. The glycosylation was performed in dichloromethane at room temperature under an inert atmosphere. The conversion of the starting material was monitored with ^{13}C NMR.

Table 1 Initial screening of halogen promoters.



Entry	Promoter	Time (h)[a]
1	I_2 (2.0 equiv.)	48h
2	NBS (1.0 equiv.)	-
3	NIS (1.5 equiv.)	3h
4	IBr (1.5 equiv.)	24h
5	$ZnBr_2$ (1.5 equiv.)	7h

Conditions: Donor (0.50 mmol), Acceptor (1.00 mmol), promoter (0.75 mmol), CH_2Cl_2, rt, [a] Full conversion of donor monitored by ^{13}C NMR.

Iodine as a promoter did not perform as well as expected based on the literature, and resulted in a long reaction time (Entry 1, Table 1). Full conversion of the starting material was only observed after 48 hours and the isolated yield of the product **125** was 73%, thus showing a significantly lower reactivity of the donor than for 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide reported by Field and co-workers[44]. This difference can be explained by influence of the protecting groups on the reactivity. Wong and co-workers[117] conducted a study on the influence of protecting groups on the reactivity for thioglycoside donors. Relative rate values were assigned to the donors carrying different protecting patterns, which were based on competition studies. Some selected pyranosides, and the corresponding relative rate values form the study are in shown Figure 8. Based on this study, a 10-fold difference in reactivity exists between 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide **(102)** and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide, assuming these differences in reactivity can be correlated directly to glycosyl halides. This might explain the insufficient activation after a prolonged reaction time.

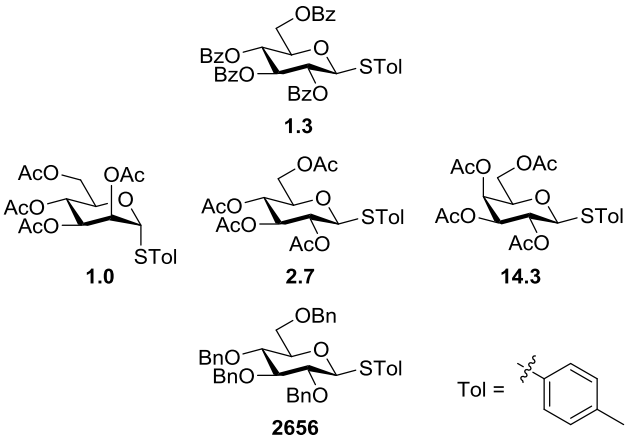


Figure 8 Protected glycopyranoside and their relative rate values obtained through competition reactions by Wong and co-workers.

Employing NBS as the promoter (Entry 2, Table 1) did not result in activation of the glycosyl donor within 24 hours. On the contrary NIS managed the activation and full conversion of the glycosyl donor **102** to the product **125** was achieved within 3 hours (Entry 3, Table 1). The resulting cyclohexyl 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside **(125)** was isolated in 95% yield.

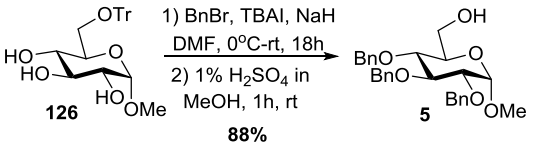
Iodine monobromide had previously displayed the ability to activate disarmed glycosyl bromides.[182] In our setup iodine monobromide (Entry 4, Table 1) performed more efficiently than iodine, although full conversion of the glycosyl donor **102** was only observed after 24 hours. Long reaction times with simple alcohols as the acceptor are not satisfactory, since the formation of the glycosidic linkage with a highly disarmed donor and more complex acceptors would then be unlikely to occur.

Zinc bromide (Entry 5, Table 1), as well as NIS, was a promising promoter, since the conversion of the starting materials to the product was achieved within 7 hours.

Of all the promoters investigated in the initial screening, NIS and zinc bromide exhibited the most promising results, and thus they were chosen for further investigation.

2.2 Optimization with reactive glycosyl acceptor

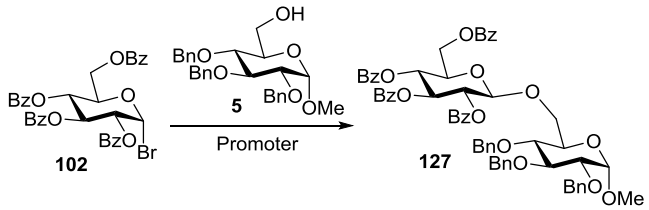
Zinc bromide and NIS were further investigated using the setup in Table 2 with the reactive monosaccharide acceptor methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **(5)**. The acceptor **5** was synthesized in one step according to a literature procedure[183] from methyl 6-*O*-trityl-α-D-glucopyranoside **(126)**[184] resulting in an overall yield of 88% (Scheme 42).



Scheme 42 Synthesis of acceptor **5**.

Iodine was also examined as a promoter with glycosyl acceptor **5**, which resulted in 10% yield (Entry 1, Table 2) after 48 hours. Zinc bromide was investigated in the same setup, but no product formation was observed according to TLC within 4 days (results not shown), and therefore zinc bromide was dismissed as a promoter for disarmed glycosyl donors.
NIS proved able to activate the disarmed donor, but the reaction was still sluggish and only resulted in a low yield (Entry 2 & 3, Table 2). Formation of the hydrolysis product of the glycosyl donor was observed in the glycosylation. No reaction was observed during the first 3 hours, which indicated difficulties with the initiation of the reaction.
The difficulty of initiating the reaction was attributed to NIS being insoluble in dichloromethane. The low solubility of NIS was circumvented using triflic acid (TfOH) and resulted in 75% isolated yield after 1 hour (Entry 4, Table 2).

Table 2 Optimization of promoter system using a reactive acceptor



Entry	Promoter System	Time (h)	Yield[a]
1	I_2 (2.0 equiv.)	48	10%
2	NIS (1.5 equiv.)	24	38%
3	NIS (2.0 equiv.)	24	36%
4	NIS (2.0 equiv.) + TfOH (1.0 equiv.)	1	75%
5	NIS (2.0 equiv.) + TfOH (0.3 equiv.)	1.5	88%
6	NIS (2.0 equiv.) + TfOH (0.1 equiv.)	3	80%
7	NIS (1.5 equiv.) + TfOH (0.15 equiv.)	1	53%
8	TfOH (0.2 equiv.)	-	-
9	NIS (2.0 equiv.) + *p*TSA (0.2 equiv.)	5	59%
10	NIS (2.0 equiv.) + CSA (0.2 equiv.)	2	71%

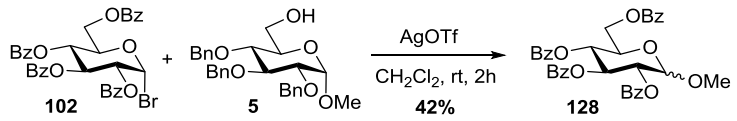
Conditions: donor (0.48 mmol), acceptor (0.32 mmol), promoter system, CH_2Cl_2, rt.
[a]Isolated yield.

Triflic acid creates harsh acidic conditions and it was therefore desirable to use catalytic amounts. Good yields were achieved under these catalytic conditions with a slight trade off in the reaction time (Entry 5 & 6, Table 2). However, decreasing the amount of NIS resulted in a significant drop in the yield (Entry 7, Table 2), even though no other products were observed by ^{13}C NMR.

Triflic acid was found in the literature[42] to be able to activate some glycosyl fluorides. To exclude TfOH as an activator of the glycosyl bromides, a catalytic amount was applied with no NIS present (Entry 8, Table 2). No activation of the donor occurred within 24 hours, and instead the acceptor reacted with itself and 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose[185] was observed.

Milder organic acids were considered for initiating the reaction in order to have slightly less acidic conditions. Both camphorsulfonic acid (CSA) and *p*-toluenesulfonic acid (*p*TSA) were each used in catalytic amounts to initiate the reaction in Table 2. For *p*TSA the isolated yield was 59% (Entry 9, Table 2) after 5 hours, which could likely be optimized, since much hydrolyzed donor was recovered along with the product. However, since pure *p*TSA is hygroscopic and highly toxic[186], it was not further pursued as an acid catalyst. Instead CSA was employed to catalyze the reaction resulting in 71% (Entry 10, Table 2) isolated yield in 2 hours for the unoptimized reaction. The lower yield was deemed a reasonable trade off, since CSA provided more mild reaction conditions applicable to more acid labile functionalities in contrary to triflic acid.

A comparison between the general activation conditions of glycosyl bromides and our promoter system was desirable. Therefore, a benchmark reaction using the silver(I) triflate[16] for activation was conducted like the setup in Table 2. The result of this glycosylation was, however, methylation of the donor, instead of glycosylation of the acceptor as expected. An anomeric mixture of methyl 2,3,4,6-tetra-*O*-benzoyl-D-glucopyranoside **128**[187] (2:5 α/β) was obtained in 42% yield (Scheme 43).



Scheme 43 Activation of glycosyl bromide with AgOTf.

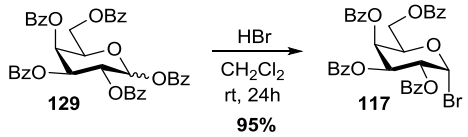
This result is suggesting a reaction of the acceptor with itself to form the 1,6-anhydrosugar, as seen when using TfOH, and furthermore liberation of methanol. However, the anhydrosugar was not identified.

2.3 Donor Scope

Donors protected with different protecting groups were then investigated, and a comparison between glucose, galactose and mannose was also done using their benzoylated glycosyl bromide equivalents.

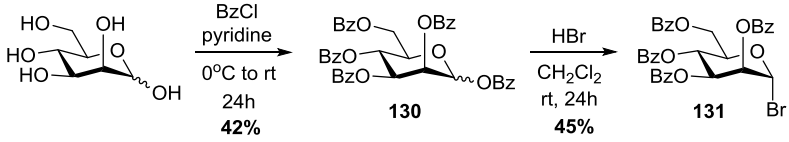
In entry 10 (Table 2), CSA was used as obtained from the supplier and not purified, hence there was room for improving this reaction. CSA was recrystallized from ethyl acetate[188] and applied in the reaction, which resulted in an increased yield of 87% (Entry 1, Table 3) indicating that CSA from supplier was insufficiently pure and dry.

First 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide **(117)** and 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide **(131)** were investigated in our setup. 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide **117** was obtained from perbenzoylated galactopyranose **129**[189], using hydrogen bromide to form the galactopyranosyl bromide **117**, in 95% yield (Scheme 44).[190]



Scheme 44 Synthesis of 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide **(117)**.

2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl bromide **(131)** was synthesized in two steps according to a literature procedure[190] from D-mannose in an overall 19% yield (Scheme 45).

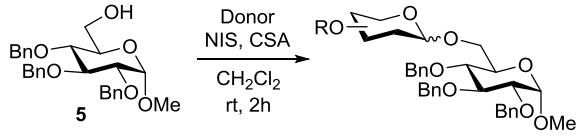


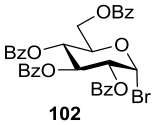
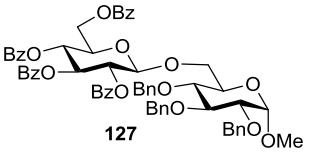
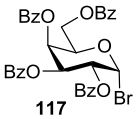
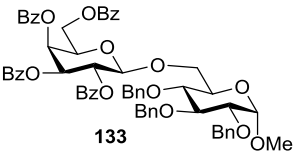
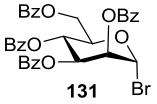
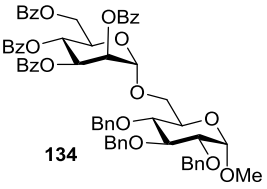
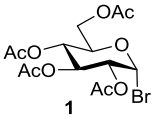
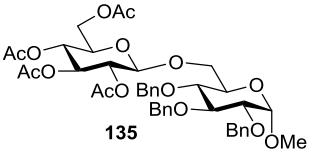
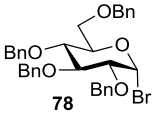
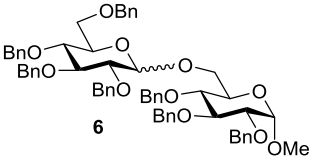
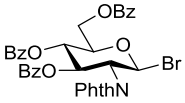
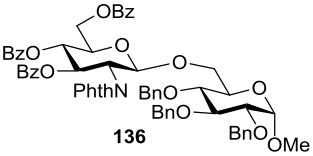
Scheme 45 Synthesis of 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide **(131)**.

Using the promoter system in Table 3 for activation of 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide **(117)** and 2,3,4,6-tetra-*O*-benzoyl-

α-D-mannopyranosyl bromide **(131)** resulted in 73% and 76% yield, respectively, of the corresponding glycosylation products **133** and **134** (Entry 2 & 3, Table 3).

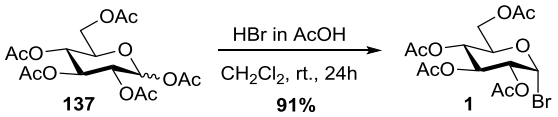
Table 3 Investigation of the promoter system using different glycosyl donors.



Entry	Donor	Product	Yield[a]
1	 **102**	 **127**	87%
2	 **117**	 **133**	73%
3	 **131**	 **134**	76%
4	 **1**	 **135**	53%
5[b]	 **78**	 **6**	63% (1:2 α/β)
6[c]	 **132**	 **136**	77%

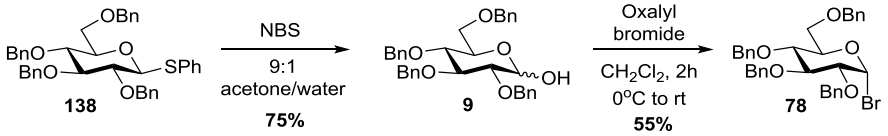
Conditions: Donor (1.5 equiv.), Acceptor (1.5 equiv.), NIS (2.0 equiv.), CSA (0.2 equiv.), CH_2Cl_2, rt, 2h. [a]Isolated yield [b]0.1 equiv. CSA, [c]1h

The promoter system was also investigated with regard to more armed donors, and both benzylated and acetylated glucopyranosyl bromides were applied. 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide **(1)** was synthesized from commercially available glucose pentaacetate **(137)** in 91% yield (Scheme 46).



Scheme 46 Synthesis of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **(1)**.

2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl bromide **(78)** was synthesized in two steps from phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside **(138)**[73]. The thioglucoside was hydrolyzed with *N*-bromosuccinimide[191], and then oxalyl bromide was used to convert 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose **(9)** into the corresponding glucopyranosyl bromide **78**[168] (Scheme 47).

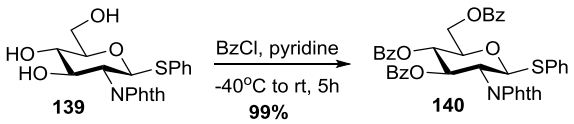


Scheme 47 Synthesis of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide **(78)**

For both 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **(1)** and 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose bromide **(78)** (Entry 4 & 5, Table 3), when applied in the setup in Table 3, the yield was significantly decreased as compared to the benzoyl protected donors. Reactions with acetyl and benzyl protected glycosyl donors (Entry 4 & 5, Table 3) afforded complex mixtures in moderate yields and purification of the products was not possible. For this reason, no further investigations of these more armed donors were made.

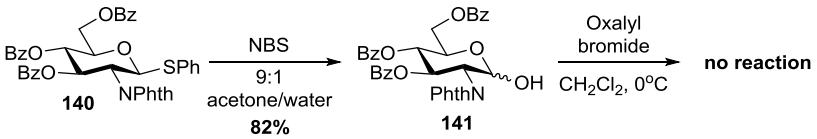
Glucosamines are found in natural products, and therefore of interest as donors in the chemical synthesis of oligosaccharides. 3,4,6-Tri-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide **(132)** has previously

been synthesized from 1,3,4,6-tetra-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranose using hydrogen bromide.[192] However, phenyl 2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside **(139)** was already available, and hence it was used as the precursor for the bromide donor. The substrate was benzoylated using benzoyl chloride in pyridine at -40°C resulting in 99% yield after 5 hours[193] (Scheme 48).



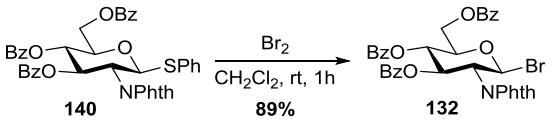
Scheme 48 Benzoylation of compound **139**.

Phenyl 3,4,6-tri-*O*-benzoyl-2-deoxy-2-phthalimido1-thio-β-D-glucopyranoside **(140)** was then hydrolyzed to the 3,4,6-tetra-*O*-benzoyl-2-deoxy-2-phthalimido-D-glucopyranose **(141)** with NBS in 9:1 acetone/water[191] in 82% yield. However, obtaining the glycosyl bromide **132** by employing oxalyl bromide was unsuccessful, since no conversion of the starting material occurred (Scheme 49).



Scheme 49 Hydrolysis and conversion to glycosyl bromide.

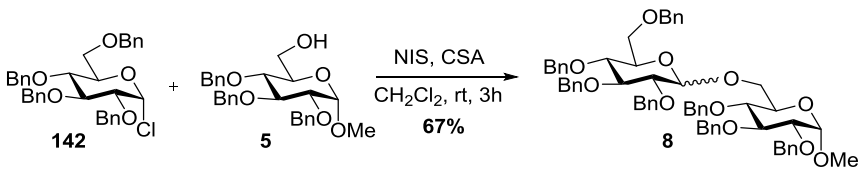
Instead compound **140** was titrated with bromine under dry conditions, resulting in 3,4,6-tri-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide **132** in 89% yield (Scheme 50). The anomeric configuration was confirmed by proton NMR through the coupling constant, $J_{1,2} = 9.5$ Hz.



Scheme 50 Synthesis of glycosyl bromide **132**.

Glycosylation of methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **(5)** with 3,4,6-tri-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide **(132)** proved to be fast compared to the other donors in Table 3. Disappearance of the starting material was observed within 1 hour (Entry 6, Table 3) giving the product in 77% yield. The seemingly high reactivity of donor **132** was ascribed to the equatorial glycosyl bromide instead of the axial glycosyl bromides otherwise used, since the 2-deoxy-2-phthalimido group does not have any significant effect on the reactivity of glycosyl donors, compared to the fully benzoylated glucopyranosyl counterpart, according to Wong *et al.*[117]

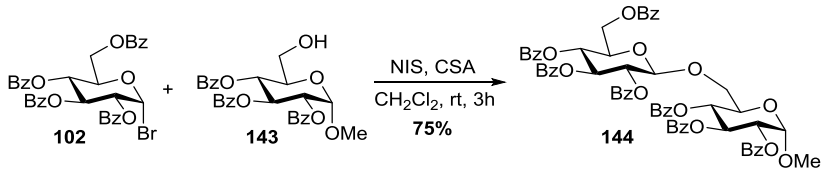
Activation of glycosyl chlorides with the promoter system was investigated in a separate bachelor project[194], since glycosyl chlorides are often grouped together with glycosyl bromides in textbooks. Benzoyl, acetyl and benzyl protected glucopyranosyl chlorides were employed. No activation of benzoyl and acetyl protected glucopyranosyl chlorides were achieved. However, the benzyl protected glycosyl chloride **(142)** afforded the product **8** in 67% in 3 hours as shown in Scheme 51, when employed in the setup from Table 3.



Scheme 51 Glycosylation employing benzyl protected glucopyranosyl chloride.

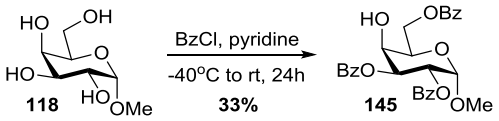
2.4 Acceptor Scope

Further investigations of the scope for our promoter system were done for acceptors with a variation in reactivity and functionalities, since the nature of the acceptor plays an important role in a glycosylation.
Methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside **(144)** was glycosylated under the same conditions as in Table 3, resulting in 75% yield (Scheme 51). 1,2,3,4,6-Penta-*O*-benzoyl-β-D-glucose was observed as a by-product in 23% yield from 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide **(102)**.



Scheme 52 Glycosylation of benzoyl protected acceptor **143**.

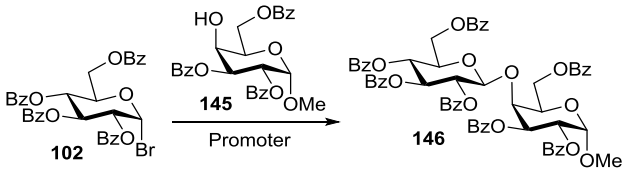
One of the more difficult alcohols to glycosylate is the axial 4-position in galactose derivatives. Therefore, investigating the ability of the promoter system with this type of unreactive acceptor was important. Methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside **(145)** was chosen for the glycosylation, since the benzoyl protecting group was found to be reasonably stable. Kozikowski *et al.*[195] established that the equatorial hydroxyl groups could be protected selectively over the axial hydroxyl groups in galactose, which allowed for the acceptor **145** to be synthesized in one step from methyl α-D-galactopyranoside **118** in 33% yield (Scheme 53).



Scheme 53 Synthesis of unreactive acceptor **145**.

Acceptor **145** was investigated in the setup shown in Table 4. In the case of CSA for catalyzing the reaction, no product formation could be observed (Entry 1, Table 4). Therefore, triflic acid was employed for the unreactive acceptor. Glycosylation of methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside **(145)** by employing triflic acid proceeded in 81% yield (Entry 2, Table 4), although the reaction time was significantly prolonged as compared to the glycosylation of the more reactive acceptor in Table 2. The product was deemed stable under the conditions, since no decomposition was observed.

Table 4 Glycosylation of unreactive galactopyranosyl acceptor **145**.



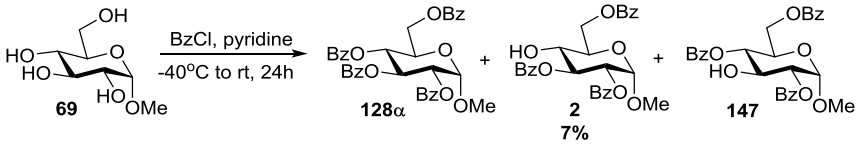
Entry	Promoter	Time	Yield[a]
1	NIS (2.0 equiv.) + CSA (0.2 equiv.)	48h	0%
2	NIS (2.0 equiv.) + TfOH (0.3 equiv.)	24h	81%
3[b]	NIS (1.5 equiv.) + TfOH (0.3 equiv.)	24h	37%
4	NIS (2.0 equiv.) + TfOH (0.1 equiv.)	24h	30%

Conditions: donor (1.5 equiv.), acceptor (1.0 equiv.), promoter, CH_2Cl_2, rt, [a]Isolated yield, [b]1.2 equiv. donor

In this setup, the acidity resulting from the use of triflic acid did not remove the protecting groups, but consideration should be given to the acid liability of other protecting groups employed when glycosylating unreactive acceptors, due to the long reaction times. Any decrease in either NIS or triflic acid lead to a significantly lower yield (Entry 3 & 4, Table 4).

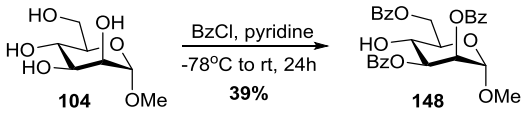
The glucopyranosyl and mannopyranosyl counterparts of the galactopyranosyl acceptor **145** were investigated as well. The equatorial secondary alcohols in the 4-position of these acceptors make them more

reactive, and a shorter reaction time was therefore expected, but the 4-position was still expected to be the least reactive position in the unprotected substrates and therefore allowing for selective protection.[196] Methyl 2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside **(2)** was prepared in one step from methyl α-D-glucoside **(69)** in 7% isolated yield from a mixture of three different products, which included the fully benzoylated product **128α**, methyl 2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside **(2)** and methyl 2,4,6-tri-*O*-benzoyl-α-D-glucopyranoside **(147)**. Separation of the two latter products proved difficult, and therefore no further separation was attempted after isolation of methyl 2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside **(2)** in sufficient quantities.



Scheme 54 Synthesis of methyl 2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside **2**.

Methyl 2,3,6-tri-*O*-benzoyl-α-D-mannopyranoside **(148)** was prepared in one step from methyl α-D-mannopyranoside **(104)** resulting in 39% yield Scheme 55.



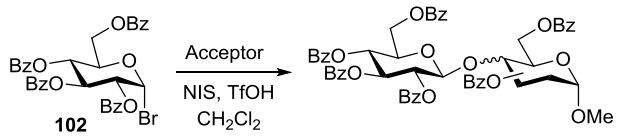
Scheme 55 Synthesis of methyl 2,3,6-tri-*O*-benzoyl-α-D-mannopyranoside **(148)**.

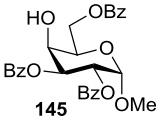
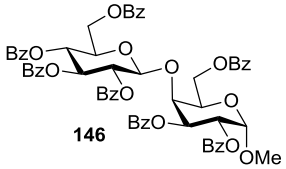
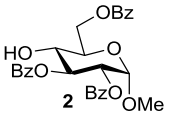
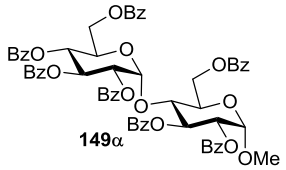
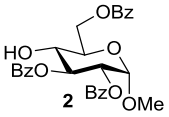
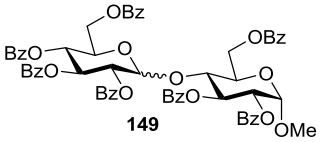
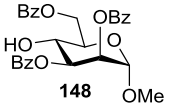
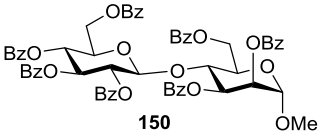
The acceptors **2** and **148** were reacted under the conditions described in Table 5. Glycosylation of **145** in entry 1 (Table 5) was added for comparison.

First acceptor **2** was glycosylated using NIS and CSA as the acid (Entry 2, Table 5), since this acceptor was assumed to be more reactive than the galactoside counterpart. Full consumption of the starting material was observed on TLC after 2 days, and 28% yield of the disaccharide **149α** was isolated. It was expected to be the β-product, but surprisingly the

α-product was isolated. Previously, the α-product was only isolated when using cyclohexanol as the acceptor (Table 1).

Table 5 Glycosylation of the 4-position in benzoylated acceptors.



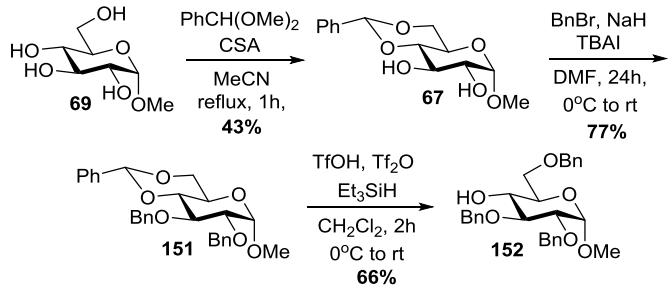
Entry	Acceptor	Time (h)	Product	Yield[a]
1[b]		24		81%
2[c]		48		28%
3		6		80% (1:2 α/β)
4		2		80%

Conditions: donor (1.5 equiv.), acceptor (1.0 equiv.), NIS (2.0 equiv.), TfOH (0.2 equiv.), CH_2Cl_2, rt, [a]Isolated yield, [b]0.3 equiv. of TfOH, [c]0.2 equiv. CSA

When substituting CSA with TfOH (Entry 3, Table 5) an anomeric mixture (ratio 1:2 α/β) of the product **149** was obtained in 80% yield. In comparison, the galactose and mannose equivalent of the acceptor afforded only the β-product in excellent yields (Entry 1 & 4, Table 5).

More reactive carbohydrate acceptors carrying secondary hydroxyl groups were of interest for further investigations. For these investigations methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside **(152)** was tested first. The acceptor **(152)** was synthesized in three steps from methyl α-D-

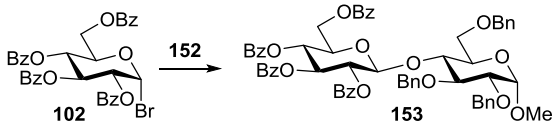
glucopyranoside **(69)** as shown in Scheme 56. Firstly, a benzylidene acetal protection of position 4 and 6 was installed,[197] followed by benzylation of the 2 and the 3 position via Williamson ether synthesis. Lastly, the benzylidene acetal was opened selectively with triethylsilane resulting in a free hydroxyl group at the 4-position.[198] All three steps gave moderate to good yields as shown in Scheme 56.



Scheme 56 Synthesis methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside **(152)**.

Glycosylation of acceptor **(152)** resulted in surprisingly low yields as shown in entry 1 (Table 6). It was speculated that the reaction did not reach completion, since TLC and NMR gave no clear picture. Therefore, increased reaction times were evaluated (Entry 2 & 3, Table 6), which just lead to a further decrease in the yields. One of the isolated products was found to be the disaccharide product with only two benzyl protecting group. 2D NMR confirmed a missing benzyl groups at the 3-position.
In an attempt to decrease the decomposition of the acceptor temperature was decreased to 0°C and the reaction stirred for 3 hours (Entry 4, Table 6). However only 21% yield was obtained. No further optimization on this substrate was attempted. In all cases, the reaction mixture was messy and purification difficult. The yields were therefore calculated from isolated mixtures.

Table 6 Glycosylation of methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside **(152)**.

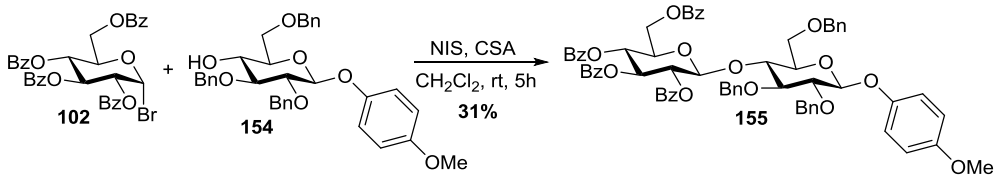


Entry	Time	Yield[a]
1	3h	40%
2	6h	33%
3	24h	27%
4[b]	3h	21%

Conditions: Donor (1.5 equiv.), Acceptor (1.0 equiv.), NIS (2.0 equiv.) + CSA (0.2 equiv.), CH_2Cl_2, rt, [a]Isolated yield, [b]0°C

Intramolecular debenzylation by NIS has been described in literature and the regioselective hereof was explained by the presence of 1,2-vicinal hydroxyl groups.[199,200] Benzyl groups are able to form benzylidene acetals in the presence of NIS, with either a light or a heat source, which in turn would likely hydrolyze in the presence of an acid and thereby explaining our observations.

Other acceptors of interest were also investigated. *p*-Methoxyphenyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside **(154)**[201] was subjected to the general conditions described in Table 3, which resulted in 31% yield (Scheme 57). This glycosylation was done prior to the experiments with the benzyl protected methyl glucose acceptor in Table 6, and the moderate yield was ascribed to decomposition of the acceptor at the anomeric center rather than benzyl deprotection of hydroxyl groups, since no acceptor could be recovered.



Scheme 57 Glycosylation of *p*-methoxyphenyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside **(154)**.

The acceptors shown in Figure 9 did not result in the desired product when subjected to the conditions in Table 3. Methyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside **(156)**[202] is a very sterically hindered acceptor. This type of acceptor has previously proven difficult to glycosylate[203] and in our case no conversion of the acceptor occurred within 3 hours.

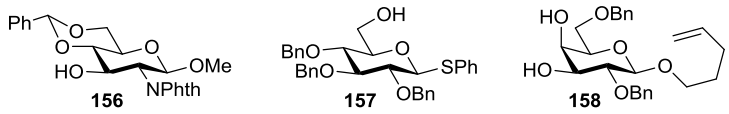
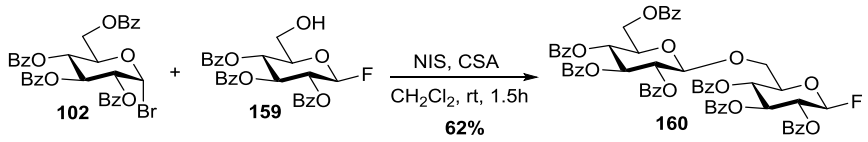


Figure 9 Acceptors unsuccessfully subjected to the conditions in Table 3.

The glycosylation of phenyl 2,3,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside **(157)**[204] did not result in product formation. Instead, the thioglycoside was activated in the presence of an iodonium ion and reacted with itself leading to formation of the 1,6-anhydrosugar[185]. Also, pentenyl 2,6-di-*O*-benzyl-β-D-galactopyranoside **(158)**[205] was available and considered a reasonable choice to investigate pentenyl glycosides under our glycosylation conditions, since galactopyranosides possess a significant difference in inherent regioselectivity between the hydroxyl group on the 3 and the 4 position. After a reaction time of 2 hours no starting material was observed, but NMR of the crude product did not reveal any alkene peaks either, which indicated decomposition of the acceptor.

Glycosyl fluorides are another commonly used halide donor and are also of interest as glycosyl acceptor, since many specific activation conditions exists. Therefore, glycosyl fluorides were a good choice for investigation, as triflic acid is known to activate this type of donor.[42] The glycosylation was done under the conditions from Table 3 using 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl fluoride **(159)**[206] as shown in Scheme 58.



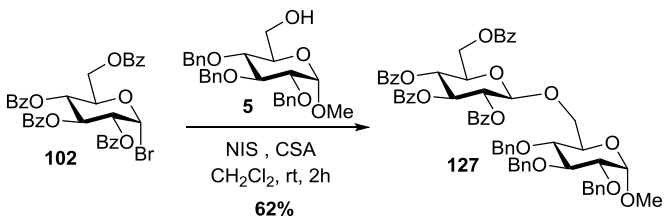
Scheme 58 Glycosylation of glycopyranosyl fluoride acceptor **159**.

The unoptimized glycosylation of acceptor **159** resulted in 62% yield, which is in agreement with the glycosylation of the similar protected acceptor methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside **(143)**.

2.5 Synthesis of *N*-iodosuccinimide

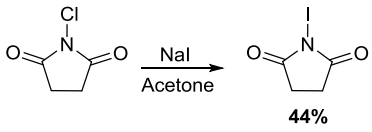
Avoiding the use of heavy metal salts as promoters in glycosylations is highly desirable, which was achieved by applying NIS and an acid for activation. NIS is commonly synthesized from the silver salt of the succinimide and iodine.[207] However, in order to investigate if heavy metal salts could be avoided all together, NIS was synthesized according to Vankar and Kumaravel.[208] In their procedure NIS can be prepared by reacting equimolar amounts of *N*-chlorosuccinimide (NCS) and NaI in acetone. The only by-product being solid NaCl is making this a convenient way to synthesize NIS.

NIS was synthesized accordingly and used directly without further purification in the glycosylation shown in Scheme 59, which resulted in 62% yield.



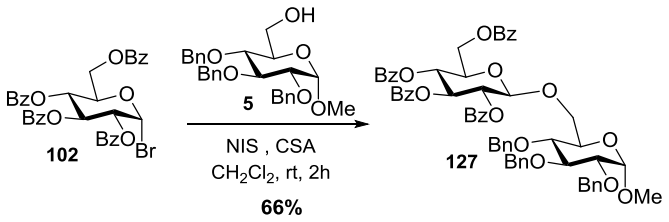
Scheme 59 Glycosylation employing synthesized NIS directly.

NIS was then synthesized according to Vankar and Kumaravel, and subsequently recrystallized from dioxane and carbon tetrachloride at -20°C[207] (Scheme 60). This was done on a 10 mmol scale instead of 1 mmol as reported by Vankar and Kumaravel, and resultet in 44% isolated yield.



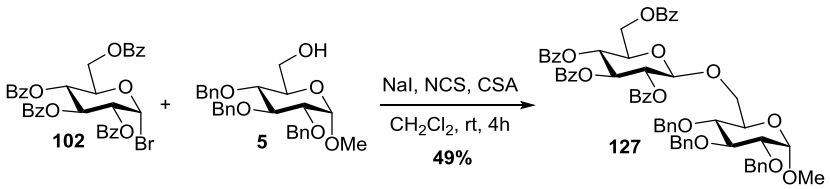
Scheme 60 Synthesis and isolation of NIS.

The purified NIS was then used in the glycosylation in Scheme 61 resulting in 66% isolated yield. The yield did not improve significantly compared to the glycosylation in Scheme 59.



Scheme 61 Glycosylation using synthesized and purified NIS.

NaCl being the only by-product formed made it an obvious choice to investigate the *in situ* formation of NIS. Therefore the acceptor **5** and the donor **102** were dissolved in dichloromethane followed addition of NaI and NCS, along with the addition of CSA (Scheme 62).

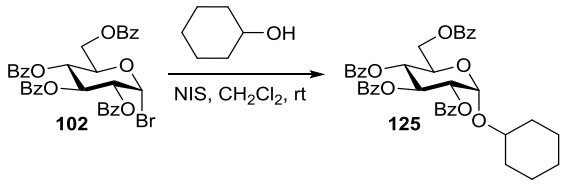


Scheme 62 Glycosylation employing *in situ* generated NIS.

The yield decreased significantly when attempting to generate NIS *in situ* as compared to Scheme 61, which is likely caused by the change of solvent. The halide substitution from NCS to NIS proceeds more efficiently in the polar solvent acetone than the dichloromethane. As a result, our glycosylation conditions performed significantly better using NIS from the supplier. However, all reactions employing NIS synthesized according to Vankar and Kumaravel are unoptimized, and therefore leaving room for improvements.

2.6 Anomerization

Anomerization of the products have been detected during the investigations of this promoter system. Therefore, investigations addressing the cause of this anomerization were performed. The most prominent case of anomerization was detected for the formation of cyclohexyl 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside **(125)** in Table 1, which due to neighboring group participation should result in cyclohexyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside **(161)**.



Scheme 63 Glycosylation of cyclohexanol with 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide employing NIS.

To investigate the formation of cyclohexyl 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside **(125)**, the reaction in Scheme 63 was followed by ^{13}C NMR as shown in Figure 10, since the anomeric carbons for both cyclohexyl 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside **(125)** and cyclohexyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside **(161)** are distinct at 94.78 ppm and 99.98 ppm, respectively. An initial mixture of both products was formed after 3 hours. The ratio of cyclohexyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside **(161)** was decreased after 5 hours and after 24 hours only cyclohexyl 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside **(125)** was identified in the reaction mixture.

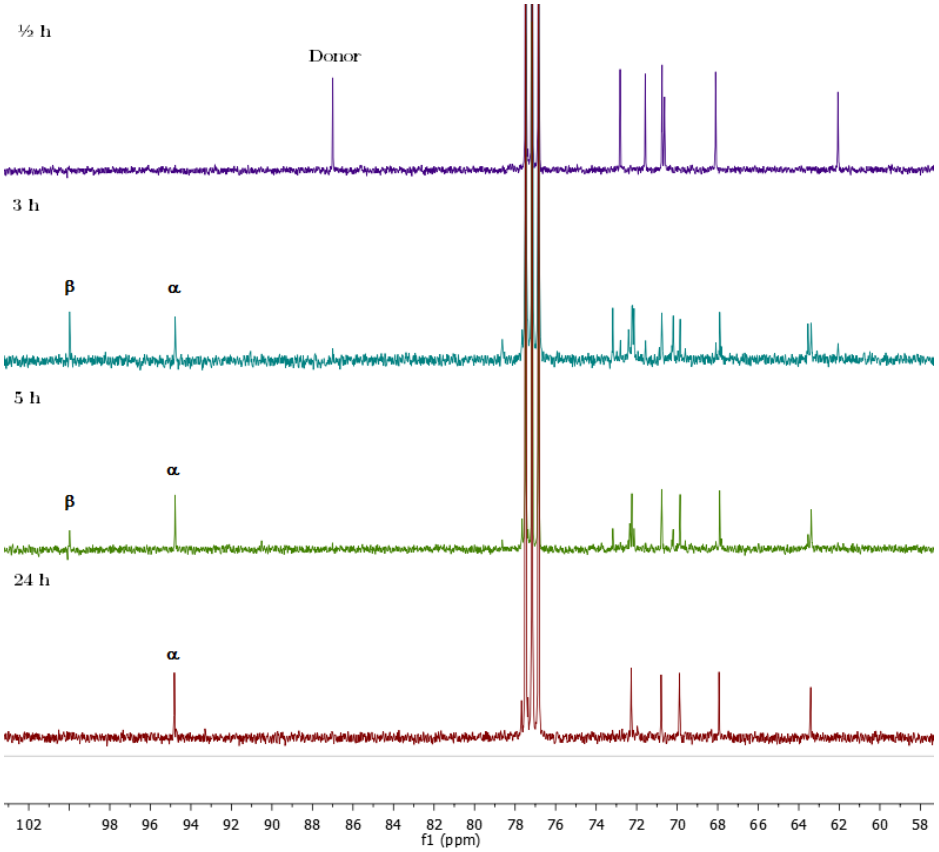
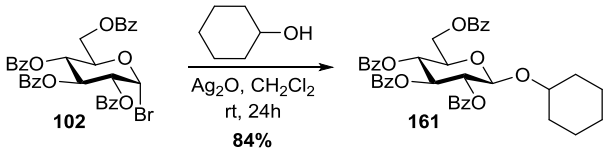


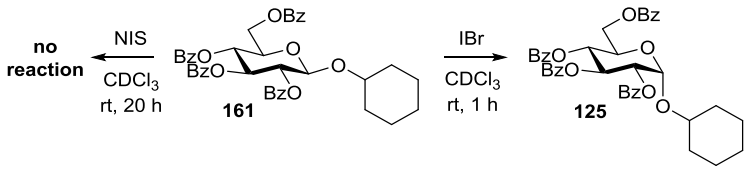
Figure 10 ^{13}C NMR monitored formation of cyclohexyl 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside for 0.5, 3, 5 and 24 hours.

Further investigation of the anomerization of cyclohexyl 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside **(125)** was done from the corresponding cyclohexyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside **(161)**, which was prepared by activation of the glycosyl bromide with silver(I) oxide[14] as shown in Scheme 64.



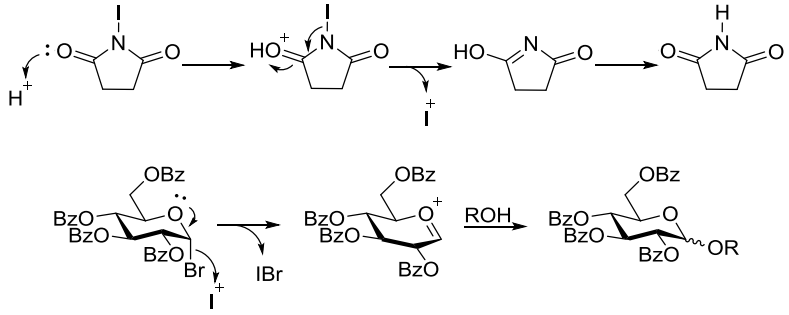
Scheme 64 Synthesis of cyclohexyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside **(161)**.

In Scheme 65, two experiments with cyclohexyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside **(161)** as substrate were then conducted. In the first experiment, cyclohexyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside **(161)** was stirred with *N*-iodosuccinimide and monitored by ^{13}C NMR, but no anomerization of the substrate occurred, which eliminates NIS as a source for the anomerization.



Scheme 65 Anomerization experiments with NIS and IBr.

The formation of iodine monobromide was expected when glycosylating with our promoter system as shown in Scheme 66, but the extent of the influence on the anomerization was not known. Therefore, an experiment with cyclohexyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside **(161)** and iodine monobromide was performed (Scheme 65). Iodine monobromide converted cyclohexyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside **(161)** into the corresponding α-product **125** within one hour, which was confirmed by ^{13}C NMR showing the anomeric carbon at 94.8 ppm.

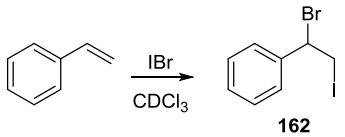


Scheme 66 Assumed mechanism of activation of glycosyl bromide with NIS.

The hydrolysis of iodine monobromide to hydrogen bromide was also investigated as a possible cause of the anomerization.[209,210] With that in mind, cyclohexyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside **(161)** was

stirred in deuterated chloroform, and an excess of hydrogen bromide in a acetic acid was added. Conversion to the α-product **125** was observed within 30 minutes, which supported the assumption of hydrogen bromide being the cause of anomerization. This is in agreement with the results of Field and co-workers, who observed the anomerization of product when using iodine, due to the formation of HI.[211] However, an excess of hydrogen bromide was necessary to facilitate anomerization. No conversion was observed using catalytic amounts, which disproved the hypothesis of hydrogen bromide causing the anomerization, since only catalytic quantities are formed with the method investigated here. Iodine and the corresponding interhalogens are able to serve as an alternative to protic acids.[212] Furthermore, iodine monochloride is known to mediate the anomerization of nucleosides.[213] Therefore, the most likely explanation is that iodine monobromide acts as a Lewis acid facilitating the anomerization.

An investigation with regard to the formation of iodine monobromide in the glycosylation was conducted. Trapping the formed iodine monobromide with an alkyne as evidence was first considered. To establish the arising peaks on ^{13}C NMR, iodine monobromide was therefore reacted with diphenylacetylene, but no conversion occurred. However, it is known that iodine monobromide reacts readily in electrophilic addition to terminal alkenes.[214] Thus, iodine monobromide was then reacted with styrene, as shown in Scheme 67, which resulted in disappearance of the alkene at 113.8 ppm and in distinct peaks at 9.5 ppm and 51.1 ppm[215] in ^{13}C NMR spectrum (Figure 11). Unfortunately, it was not possible to isolate 1-bromo-2-iodo-1-phenylethane **(162)**, due to decomposition hereof.



162

Scheme 67 Reaction of styrene with iodine monobromide.

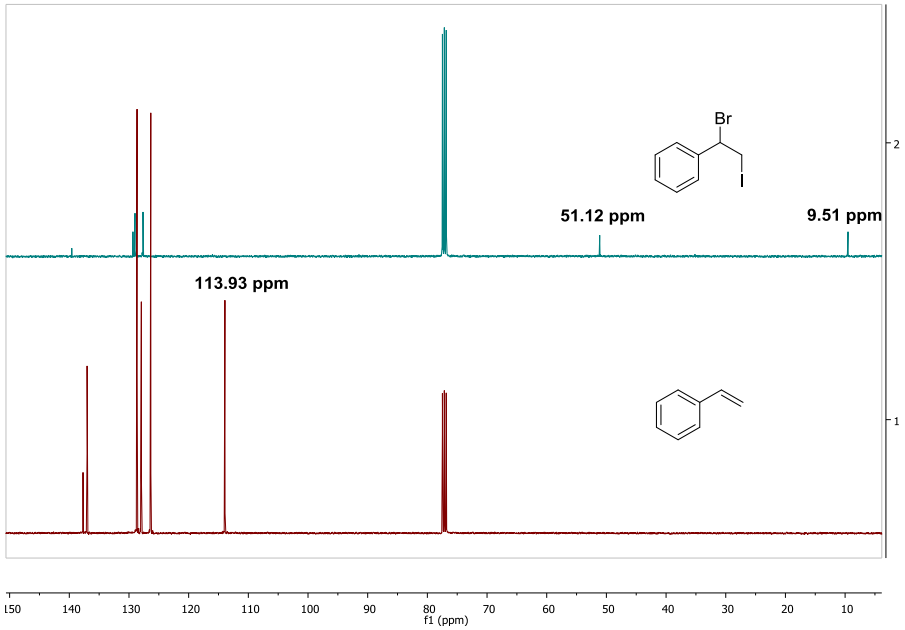


Figure 11 ^{13}C NMR of reaction of IBr with styrene.

Cyclohexanol was glycosylated with 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide **(102)** using NIS in CH_2Cl_2 at room temperature for 5 hours, creating an anomeric mixture of products as shown in Figure 12. The reaction was then quenched by adding styrene and evidence supporting the formation of iodine monobromide was found, when the peaks at 9.5 ppm and 51.1 ppm were observed as in Figure 12.

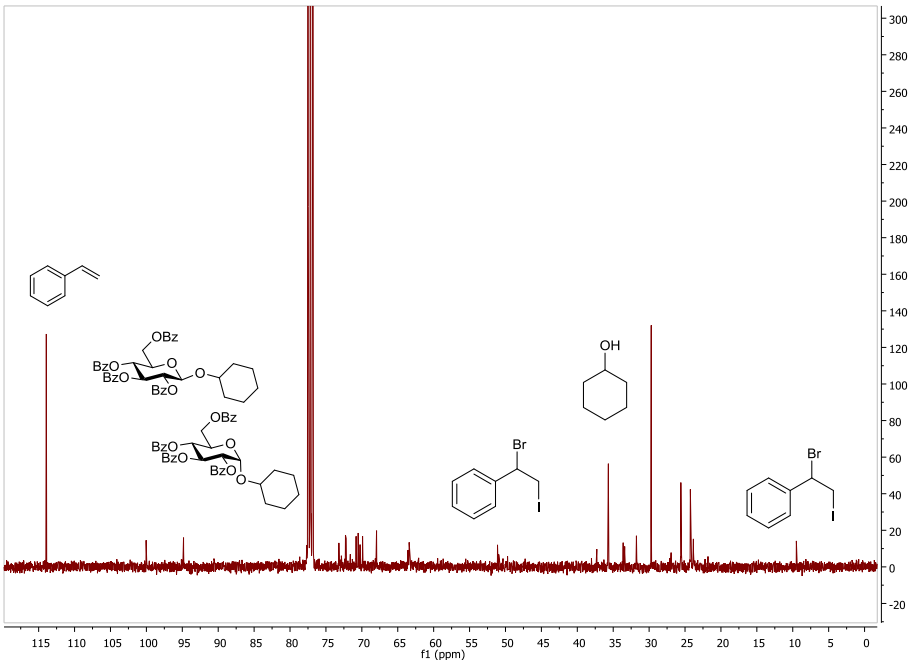


Figure 12 Cyclohexanol was glycosylated with 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide **(102)** and the reaction was quenched with styrene after 5 hours. An anomeric mixture of cyclohexyl 2,3,4,6-tetra-*O*-benzoyl-D-glucopyranoside was observed together with 1-bromo-2-iodo-1-phenylethane **(162)**.

2.7 Concluding Remarks

The investigations of the iodonium ion promoted activation of disarmed glycosyl bromides lead to the promoter system NIS together with a protic acid. For the more reactive acceptors the glycosylation could be facilitated with catalytic CSA and for the unreactive acceptors triflic acid was employed.

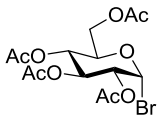
Our promoter system was generally applicable for benzoyl protected glycosyl bromides in good yields, but resulted in moderate yields for more reactive glycosyl bromides. The best result was obtained for the unreactive methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside **(145)**, which was glycosylated in 81% yield. Furthermore, NIS and CSA was applicable with glycosyl fluorides as the acceptor.

Iodine monobromide was assumed to be formed during the activation, which was proven experimentally. Furthermore, iodine monobromide was found to cause anomerization of simple β-glycosides by acting as a Lewis acid.

2.8 Experimental

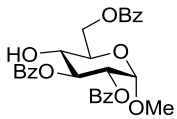
All solvents were of analytical HPLC grade and reagents were used without further purification as obtained from Sigma-Aldrich, unless otherwise noted. Anhydrous solvents were obtained from the PureSolv™ system unless otherwise stated. Cyclohexanol was distilled prior to use. Camphorsulfonic acid was recrystallized in dry EtOAc. NCS was recrystallized from glacial acetic acid[216] and acetone[217] was dried with calcium sulfate, then distillated and stored under nitrogen atmosphere. Reactions were conducted under an argon or a nitrogen atmosphere. Drying of organic layers was done with $MgSO_4$. TLC was performed on aluminum plates coated with silica gel 60. Visualization was carried out by UV and Cerium Sulfate stain. Flash column chromatography was performed with silica gel (35–60 µm) and dry column vacuum chromatography (DCVC)[218] was performed with silica gel (15–40 µm). NMR spectra were recorded with Bruker Ascend 400 spectrometer at 400 MHz and 101 MHz. Chemical shifts are measured relative to the residual solvent signal in $CDCl_3$ (δH = 7.26 ppm, δC = 77.0 ppm). Further assignment of ^{1}H and ^{13}C resonances were based on COSY, HSQC and HMBC experiments. HRMS was performed on a Bruker SolariX XR ESI/MALDI-FT-ICR-MS instrument equipped with a 7 T magnet. The instrument was run in the MALDI mode and externally calibrated with sodium trifluoroacetate cluster ions.

General procedure: Donor (1.5 equiv.) and acceptor (1.0 equiv.) were stirred in dry CH_2Cl_2 (2.0 ml) at room temperature under an inert atmosphere, followed by addition of NIS (2.0 equiv.) and catalytic amounts of the acid (0.1-0.3 equiv.). The reaction mixture was stirred until consumption of the starting material according to TLC. The reaction mixture was evaporated onto silica or diluted with CH_2Cl_2, washed with aq. sat. sodium thiosulfate and water, dried and the solvent was removed *in vacuo*, then purified by DCVC or flash column chromatography.

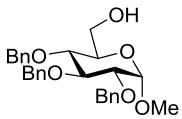


2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (1):[219] 1,2,3,4,6-Penta-*O*-acetyl-D-glucopyranose (77 mmol) in was dissolved in CH_2Cl_2 (300 ml) and 33% hydrogen bromide in acetic acid (80 ml) was added. The reaction mixture was stirred at room temperature overnight, and then ice water was added. The organic layer was washed with aq. sat. $NaHCO_3$ and water, then dried, filtered and the solvent removed *in vacuo*. The residue was dissolved in Et_2O and crystallized by addition of hexane, affording the bromide as a white solid in 91% yield. **^{1}H NMR**[219] (400 MHz, $CDCl_3$) δ 6.61 (d, J = 4.0 Hz, 1H),

5.56 (t, J = 9.7 Hz, 1H), 5.16 (t, J = 9.8 Hz, 1H), 4.84 (dd, J = 10.0, 4.1 Hz, 1H), 4.35 – 4.27 (m, 2H), 4.15 – 4.10 (m, 1H), 2.10 (s, 3H), 2.10 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 170.7, 170.0, 169.9, 169.6, 86.7, 72.3, 70.7, 70.3, 67.3, 61.1, 20.8 (3xC), 20.7.

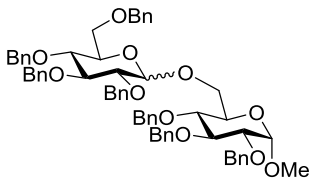


Methyl 2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside (2):[196] Benzoyl chloride (170.0 mmol) was added dropwise over 45 min to the mixture of methyl α-D-glucopyranoside (51.5 mmol) in pyridine (100 ml) at -40°C. The reaction mixture was stirred overnight at room temperature. Water (15 mL) was added and the mixture was stirred for an additional 15 min, followed by removal of all solvent *in vacuo*. The residue was dissolved in CH_2Cl_2 (50 ml) and washed with 2 M HCl, sat. aq. $NaHCO_3$ and brine. The organic layer was dried, filtered and concentrated. The reaction mixture was purified by column chromatography (EtOAc/Hexane gradient), to give 7% of the pure product. **^{1}H NMR**[220] (400 MHz, $CDCl_3$) δ 8.12 – 8.09 (m, 2H), 8.00 – 7.96 (m, 4H), 7.60 (t, J = 7.4 Hz, 1H), 7.53 – 7.46 (m, 4H), 7.40 – 7.34 (m, 4H), 5.78 (t, J = 9.7 Hz, 1H), 5.28 (dd, J = 10.2, 3.7 Hz, 1H), 5.15 (d, J = 3.6 Hz, 1H), 4.81 (dd, J = 12.2, 4.5 Hz, 1H), 4.63 (dd, J = 12.1, 2.1 Hz, 1H), 4.12 (ddd, J = 9.9, 4.3, 2.1 Hz, 1H), 3.88 (td, J = 9.6, 4.7 Hz, 1H), 3.46 (d, J = 6.2 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 167.5, 167.1, 166.1, 133.6, 133.5 (2xC), 130.0-128.4 aromatic region, 97.3, 74.1, 71.4, 70.2, 69.9, 63.6, 55.6.

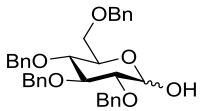


Methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (5):[183] Methyl 6-*O*-trityl-α-D-glucopyranoside (34.4 mmol) was dissolved in dry DMF (120 ml) along with NaH (147.8 mmol, 50% mineral oil). The mixture was stirred for 20 minutes, then benzyl bromide (171.8 mmol) and TBAI (0.3 mmol) were added at 0°C. The reaction was stirred at room temperature for 18 hours, and then the reaction was diluted with EtOAc and washed with brine. The organic layer were dried and removed *in vacuo*. The fully protected crude was dissolved in (1 g/20 ml) MeOH containing 1% of H_2SO_4 and stirred at room temperature for 1 hour until complete cleavage of the trityl group. The reaction mixture was stirred with Na_2CO_3 until neutral pH was achieved. The mixture was filtered and concentered, then dissolved in CH_2Cl_2, and washed twice with brine. The organic layer was dried and removed *in vacuo*. The crude product was purified by DCVC (EtOAc in hexane) resulting

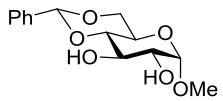
in a white solid in 88% yield. **^{1}H NMR**[221] (400 MHz, $CDCl_3$) δ 7.37 – 7.22 (m, 15H), 5.00 (d, J = 10.9 Hz, 1H), 4.89 – 4.76 (m, 3H), 4.68 – 4.60 (m, 2H), 4.57 (d, J = 3.5 Hz, 1H), 4.01 (t, J = 9.3 Hz, 1H), 3.77 (dd, J = 11.5, 2.5 Hz, 1H), 3.78 – 3.61 (m, 2H), 3.54 – 3.46 (m, 2H), 3.37 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 138.9, 138.3, 138.2, 129.2-127.8 (aromatic region), 98.3, 82.1, 80.1, 75.9, 75.2, 73.6, 70.8, 62.0, 55.3.



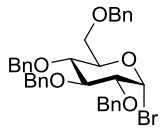
Methyl 2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl-(1,6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (6): General Procedure with CSA (0.1 equiv.) and 2 hours reaction time. Purification by column chromatography (1:4:4 EtOAc/Hexane/Toluene) resulting in an α/β mixture (1:3) in 61 % yield. **^{1}H NMR**[222] (400 MHz, $CDCl_3$) δ 7.35 – 7.09 (m, 46.66H), 4.99 – 4.94 (m, 2.66H), 4.93 – 4.86 (m, 1.33H), 4.84 – 4.48 (m, 16H), 4.45 (d, J = 11.0 Hz, 0.33H), 4.41 (d, J = 12.1 Hz, 0.33H), 4.34 (d, J = 7.8 Hz, 1H), 4.18 (dd, J = 10.8, 1.9 Hz, 1H), 3.99 (t, J = 9.3 Hz, 1H), 3.97 (d, J = 9.3 Hz, 0.33H), 3.85 – 3.38 (m, 13.33H), 3.34 (s, 1H), 3.32 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 139.0 (2xC), 138.9, 138.6 (2xC), 138.5 (3xC), 138.4, 138.3 (2xC), 138.2, 138.1, 128.6-127.6, 103.9, 98.1, 98.2, 97.4, 84.9, 82.3, 82.2, 82.1, 81.8, 80.3, 80.1, 79.9, 78.1, 78.0, 77.9, 77.7, 75.8 (3xC), 75.6, 75.1 (2xC), 75.00 (3xC), 73.6, 73.5 (3xC), 72.5, 70.5, 70.4, 70.0, 69.1, 68.7, 68.6, 66.2, 55.3 (2xC).



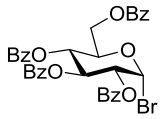
2,3,4,6-Tetra-*O*-benzyl-D-glucopyranoside (9):[191] NBS (2.6 mmol) was added to a solution of phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside in 9:1 acetone-water (15 ml/1mmol) at room temperature. Stirring was continued for a period of 10 min. The solvent was evaporated and the residue was dissolved in EtOAc. The organic layer was washed with sat. aq. $NaHCO_3$ and water, then dried and the solvent removed *in vacuo*. The product was isolated by column chromatography (1:4:4 EtOAc/Hexane/Toluene) resulting in 75% yield as an α/β mixture (5:2). NMR was according to literature.[223] **HSQC** ($CDCl_3$) δ 5.24/91.2, 4.73/97.5.



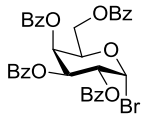
Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (67):[197] A suspension of methyl α-D-glucopyranoside (51.5 mmol), benzaldehyde dimethyl acetal (66.9 mmol) and CSA (3.4 mmol) in dry MeCN (50 ml) was heated to reflux and stirred 1 hour, and then neutralized by addition of Et_3N. The reaction was diluted with EtOAc and then washed with water and dried. The solvent was evaporated and the residue dissolved in CH_2Cl_2, then crystallized by slow addition of hexane resulting in 43% yield. **^{1}H NMR**[197] (400 MHz, $CDCl_3$) δ 7.51 – 7.48 (m, 2H), 7.39 – 7.35 (m, 3H), 5.53 (s, 1H), 4.80 (d, J = 3.9 Hz, 1H), 4.30 (dd, J = 9.6, 4.3 Hz, 1H), 3.93 (t, J = 9.2 Hz, 1H), 3.85 – 3.71 (m, 2H), 3.63 (dd, J = 9.2, 4.0 Hz, 1H), 3.50 (t, J = 9.3 Hz, 1H), 3.46 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 129.4, 128.5, 126.4, 102.1, 99.9, 81.1, 73.0, 71.9, 69.1, 62.5, 55.7.



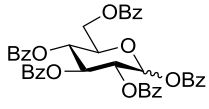
2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl bromide (78):[168] 2,3,4,6-Tetra-*O*-benzyl-D-glucopyranose (1.66 mmol) was dissolved in dry CH_2Cl_2 (4 ml) under a nitrogen atmosphere. The solution was cooled to 0°C, and then oxalyl bromide (6.66 mmol, 2 M solution in CH_2Cl_2) was added dropwise. The reaction was allowed to attain room temperature and stirred for 2 h. The reaction was cooled to 0°C and quenched with ice water. The mixture was partitioned between CH_2Cl_2 and water. The aqueous layer was extracted with CH_2Cl_2, and the combined organic layers was washed with brine, dried, filtered and concentrated *in vacuo*. The residue was purified with column chromatography (1:4:4 EtOAc/Hexane/Toluene). The product was obtained in 55% yield. **^{1}H NMR**[222] (400 MHz, $CDCl_3$) δ 8.03 – 8.00 (m, 4H), 7.77 – 7.71 (m, 2H), 7.65 – 7.59 (m, 4H), 7.40 – 7.13 (m, 10H), 6.43 (d, J = 3.7 Hz, 1H), 4.98 (d, J = 10.8 Hz, 1H), 4.86 – 4.81 (m, 2H), 4.71 (s, 2H), 4.60 – 4.44 (m, 3H), 4.08 – 4.00 (m, 2H), 3.81 – 3.74 (m, 2H), 3.65 (dd, J = 11.0, 1.9 Hz, 1H), 3.54 (dd, J = 9.2, 3.7 Hz, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 138.6, 138.1, 137.7, 137.5, 135.3, 129.7-126.6 (aromatic region), 92.0, 82.3, 79.8, 76.2, 76.0, 75.4, 75.3, 73.6, 72.9, 67.7.



2,3,4,6-Tetra-*O*-benzoyl-α-D-glucopyranosyl bromide (102):[181] 1,2,3,4,6-penta-*O*-benzoyl-D-glucopyranoside was dissolved in CH_2Cl_2 (200 ml) and 33% hydrogen bromide in acetic acid (30 ml) was added at 0°C. The reaction mixture was stirred at room temperature for 3 hours, and then ice cold water (200 ml) was added. The organic layer was washed with aq. sat. $NaHCO_3$ and water, then dried, filtered and removed *in vacuo*. The residue was crystallized from Et_2O affording a white solid in 68% yield. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.09 – 8.05 (m, 2H), 8.02 – 7.98 (m, 2H), 7.97 – 7.93 (m, 2H), 7.89 – 7.85 (m, 2H), 7.60 – 7.28 (m, 12H), 6.87 (d, J = 4.0 Hz, 1H), 6.27 (t, J = 9.8 Hz, 1H), 5.82 (t, J = 10.0 Hz, 1H), 5.33 (dd, J = 10.0, 4.0 Hz, 1H), 4.74 (ddd, J = 10.2, 4.2, 2.8 Hz, 1H), 4.67 (dd, J = 12.5, 2.6 Hz, 1H), 4.52 (dd, J = 12.5, 4.5 Hz, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.2, 165.7, 165.4, 165.2, 134.0, 133.8, 133.5, 133.4, 130.2-128.5 (aromatic region), 87.0, 72.9, 71.6, 70.8, 68.1, 62.1.

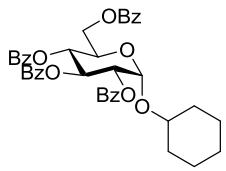


2,3,4,6-Tetra-*O*-benzoyl-α-D-galactopyranosyl bromide (117):[181] 1,2,3,4,6-Penta-*O*-benzoyl-D-galactopyranose (14.3 mmol) was dissolved in CH_2Cl_2 (80 ml) and 33% hydrogen bromide in acetic acid (16.6 ml) was added. The reaction was stirred at room temperature under an argon atmosphere overnight, and then ice water (30 ml) was added. The organic layer was washed with sat. aq. $NaHCO_3$ and water, then dried, filtered and removed *in vacuo*. The residue was crystallized from Et_2O affording a white solid in 95%. **^{1}H NMR**[224] (400 MHz, $CDCl_3$) δ 8.09 – 7.99 (m, 6H), 7.80 – 7.78 (m, 2H), 7.61 – 7.37 (m, 10H), 7.29 – 7.23 (m, 2H), 6.98 (d, J = 4.0 Hz, 1H), 6.12 (d, J = 3.2 Hz, 1H), 6.06 (dd, J = 10.4, 3.3 Hz, 1H), 5.67 (dd, J = 10.4, 4.0 Hz, 1H), 4.92 (t, J = 6.4 Hz, 1H), 4.64 (dd, J = 11.5, 6.8 Hz, 1H), 4.47 (dd, J = 11.6, 6.0 Hz, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.1, 165.7, 165.5 (2xC), 133.9, 133.5 (2xC), 130.2-128.5 (aromatic region), 88.4, 72.0, 69.0, 68.7, 68.2, 61.8.

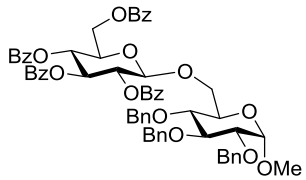


1,2,3,4,6-Penta-*O*-benzoyl-β-D-glucopyranose (124):[181] Benzoyl chloride (1.0 mol) was added dropwise over 45 min to a mixture of D-glucose monohydrate (0.1 mol) in

pyridine (200 ml) at 0°C. The reaction mixture was stirred overnight at room temperature. Water (70 ml) was added and the mixture was stirred for an additional 45 min, and then pyridine was removed *in vacuo*. The residue was dissolved in CH_2Cl_2 and the organic layer was washed with 2 M HCl, aq. sat. $NaHCO_3$ and water. The organic layer was dried, filtered and concentrated *in vacuo*. The residue was purified by recrystallization from EtOAc/hexane to afford a white solid in 83% yield. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.05 (d, J = 7.7 Hz, 4H), 7.92 – 7.84 (m, 6H), 7.58 – 7.25 (m, 15H), 6.32 (d, J = 7.9 Hz, 1H), 6.06 (t, J = 9.4 Hz, 1H), 5.92 – 5.81 (m, 2H), 4.67 (dd, J = 12.3, 2.2 Hz, 1H), 4.52 (dd, J = 12.2, 4.6 Hz, 1H), 4.46 – 4.38 (m, 1H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 166.2, 165.8, 165.3 (2xC) 164.7, 134.0, 133.7, 133.6, 133.5, 133.2, 130.3-128.5 (aromatic region), 92.8, 73.3, 72.9, 70.9, 69.1, 62.8.

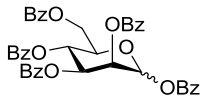


Cyclohexyl 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside (125): Cyclohexanol (1.00 mmol) and 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide (0.50 mmol) were stirred in dry CH_2Cl_2 (2 ml) at room temperature under inert atmosphere, followed by addition of the promoter (0.75-1.00 mmol). After 1-28 hours full conversion to cyclohexyl 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside was observed. The solvent was evaporated and the residue was purified by column chromatography (6:1 Toluene/Acetone). **^{1}H NMR**[225] (400 MHz, $CDCl_3$) δ 8.10 – 8.05 (m, 2H), 8.03 – 7.94 (m, 4H), 7.91 – 7.87 (m, 2H), 7.55 – 7.25 (m, 12H), 6.22 (t, J = 9.9 Hz, 1H), 5.67 (t, J = 9.9 Hz, 1H), 5.52 (d, J = 3.8 Hz, 1H), 5.29 (dd, J = 10.2, 3.8 Hz, 1H), 4.63 – 4.57 (m, 2H), 4.52 – 4.42 (m, 1H), 3.69 – 3.56 (m, 1H), 2.04 – 1.91 (m, 1H), 1.78 – 1.39 (m, 5H), 1.38 – 1.07 (m, 4H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.3, 166.0 (2xC), 165.5, 133.5-128.3 (aromatic region), 94.8, 77.7, 72.3, 70.8, 69.9, 68.0, 63.4, 33.6, 31.7, 25.6, 24.1, 23.8.

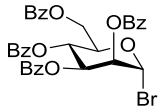


Methyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1,6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (127): General Procedure with CSA (0.2 equiv.) and reaction time of 2 hours. Purification by column chromatography (5% acetone in toluene) resulting in 87%

yield. **^{1}H NMR**[226] (400 MHz, $CDCl_3$) δ 8.02 – 7.98 (m, 2H), 7.94 – 7.88 (m, 4H), 7.88 – 7.81 (m, 2H), 7.56 – 7.01 (m, 27H), 5.91 (t, J = 9.6 Hz, 1H), 5.70 (t, J = 9.7 Hz, 1H), 5.62 (dd, J = 9.7, 7.8 Hz, 1H), 4.91 (d, J = 10.9 Hz, 1H), 4.84 (d, J = 7.8 Hz, 1H), 4.75 (d, J = 11.5 Hz, 1H), 4.69 (d, J = 11.5 Hz, 1H), 4.63 (dd, J = 12.0, 3.4 Hz, 1H), 4.61 (d, J = 12.1 Hz, 1H), 4.58 – 4.48 (m, 3H), 4.31 (d, J = 11.2 Hz, 1H), 4.17 (d, J = 9.1 Hz, 1H), 4.12 (ddd, J = 13.4, 5.9, 3.3 Hz, 1H), 3.90 (t, J = 9.3 Hz, 1H), 3.80 – 3.69 (m, 2H), 3.45 (dd, J = 9.7, 3.5 Hz, 1H), 3.40 (t, J = 9.3 Hz, 1H), 3.23 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.2, 165.9, 165.2, 165.0, 138.9, 138.3, 138.2, 137.9, 134.0, 133.6, 133.5, 133.3, 133.2 (2xC), 130.1-127.5 (aromatic region), 101.4, 98.0, 92.3, 90.5, 81.9, 79.8, 75.6, 74.8, 73.4, 72.9, 72.3, 71.9, 69.9, 69.5, 68.4, 63.3, 55.1.

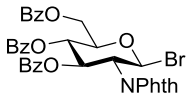


1,2,3,4,6-Penta-*O*-benzoyl-D-mannopyranose (130):[181] Benzoyl chloride (111.0 mmol) was added dropwise to D-mannopyranose (11.1 mmol) in pyridine (5 ml) at °C. The reaction mixture was stirred overnight at room temperature. Water was added and the mixture was stirred for an additional 30 min and then the solvent was removed *in vacuo*. The residue was dissolved in CH_2Cl_2 and washed with 2 M HCl, aq. sat. $NaHCO_3$ and water. The organic layer was dried, filtered and concentrated *in vacuo*. The residue was dissolved in EtOAc and crystallized by addition of hexane, resulting in an anomeric mixture as an amorphous solid in 42 % yield. α anomer: **^{1}H NMR**[227] (400 MHz, $CDCl_3$) δ 8.25 – 7.14 (m, 25H), 6.64 (d, J = 1.7 Hz, 1H), 6.30 (t, J = 10.1 Hz, 1H), 6.08 (dd, J = 10.2, 3.3 Hz, 1H), 5.94 – 5.92 (m, 1H), 4.71 (dd, J = 12.3, 2.4 Hz, 1H), 4.64 – 4.47 (m, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.2 – 164.0 (C=O), 134.7 – 128.5 (aromatic region), 91.5, 71.3, 70.1, 69.6, 66.3, 62.5. β anomer: **^{1}H NMR**[227] (400 MHz, $CDCl_3$) δ 8.25 – 7.14 (m, 25H), 6.45 (s, 1H), 6.19 (t, J = 9.8 Hz, 1H), 6.12 (d, J = 3.0 Hz, 1H), 5.82 (dd, J = 9.9, 3.1 Hz, 1H), 4.77 (dd, J = 12.3, 2.6 Hz, 1H), 4.64 – 4.47 (m, 1H), 4.38 (dt, J = 9.7, 3.5 Hz, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.2 – 164.0 (C=O), 134.7 – 128.5 (aromatic region), 91.4, 73.5, 71.7, 69.6, 69.5, 62.8.

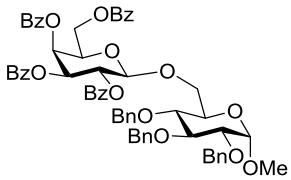


2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl bromide (131):[181] 1,2,3,4,6-penta-*O*-benzoyl-D-mannopyranose in was dissolved in CH_2Cl_2 (30 ml) and 33% hydrogen bromide in acetic acid (5.5 ml) was added. The reaction mixture was stirred at room temperature overnight, then ice water (30 ml) was added. The organic layer was washed with sat. aq.

$NaHCO_3$ and water, then dried, filtered and removed *in vacuo*. The residue purified by column chromatography (Toluene/Acetone 9:1) affording the bromide as a white candyfloss in 45% yield. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.13 – 8.09 (m, 2H, Ar), 8.04 – 8.02 (m, 2H, Ar), 8.01 – 7.97 (m, 2H, Ar), 7.87 – 7.83 (m, 2H, Ar), 7.64 – 7.50 (m, 3H, Ar), 7.48 – 7.36 (m, 7H, Ar), 7.29 – 7.25 (m, 2H, Ar), 6.59 (d, J = 1.2 Hz, 1H, 1-H), 6.31 – 6.22 (m, 2H, 3-H, 4-H), 5.92 (dd, J = 2.9, 1.7 Hz, 1H, 2-H), 4.75 (dd, J = 12.5, 2.4 Hz, 1H, 6a-H), 4.68 – 4.64 (m, 1H, 5-H), 4.52 (dd, J = 12.5, 3.8 Hz, 1H, 6b-H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.1 (C=O), 165.5 (C=O), 165.4 (C=O), 165.1 (C=O), 133.9, 133.8, 133.5, 133.3, 130.0-128.4 (aromatic region), 83.4 (C-1), 73.6 (C-5), 73.1 (C-2), 69.2 (C-3), 66.1 (C-4), 61.9 (C-6). **HRMS** (MALDI) calcd for $C_{34}H_{27}BrO_9$ $[M+Na]^+$ m/z 681.0731, found 681.0749.

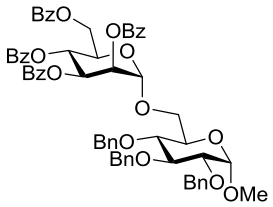


3,4,6-Tri-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (132): Phenyl 3,4,6-tri-*O*-benzoyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (1.19 mmol) was dissolved in dry CH_2Cl_2 (9 ml) and stirred under a nitrogen atmosphere at room temperature. Bromine (1.19 mmol) in dry CH_2Cl_2 (2 ml) was added dropwise. The reaction was stirred for 1 hour after the addition. The mixture was diluted with CH_2Cl_2 and poured into sat. aq. sodium thiosulfate. The organic layer was separated, washed with water, dried and removed *in vacuo*. The residue was purified by column chromatography (3:10 EtOAc/Hexane) resulting in 89% yield. **^{1}H NMR**[192] (400 MHz, $CDCl_3$) δ 8.09 – 8.05 (m, 2H), 7.92 – 7.88 (m, 2H), 7.86 – 7.68 (m, 6H), 7.59 – 7.23 (m, 9H), 6.64 (d, J = 9.5 Hz, 1H), 6.28 (dd, J = 10.3, 9.4 Hz, 1H), 5.83 (t, J = 9.7 Hz, 1H), 4.92 (dd, J = 10.3, 9.7 Hz, 1H), 4.68 (dd, J = 12.4, 2.8 Hz, 1H), 4.54 (dd, J = 12.4, 4.9 Hz, 1H), 4.38 (ddd, J = 10.1, 4.8, 2.9 Hz, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.2, 165.7, 165.2, 134.6, 133.7, 133.6, 133.4, 131.4-128.4 (aromatic region), 124.0, 77.7, 77.2, 71.2, 69.4, 62.9, 58.6.

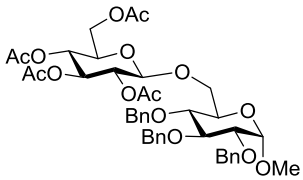


Methyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1,6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (133): General procedure with CSA (0.2 equiv.) and reaction time of 2 hours. Purification by column chromatography (3% acetone in toluene) resulting in 73% yield. **^{1}H NMR**[228] (400 MHz, $CDCl_3$) δ 8.10 (d, J = 7.6 Hz, 2H), 8.03 (d, J = 7.6 Hz, 2H),

7.90 (d, J = 7.7 Hz, 2H), 7.78 (d, J = 7.7 Hz, 2H), 7.70 – 6.99 (m, 27H), 5.99 (s, 1H), 5.86 (t, J = 9.1 Hz, 1H), 5.61 (d, J = 10.4 Hz, 1H), 4.91 (d, J = 10.9 Hz, 1H), 4.79 – 4.65 (m, 4H), 4.64 – 4.54 (m, 2H), 4.52 (d, J = 3.5 Hz, 1H), 4.46 – 4.34 (m, 2H), 4.30 – 4.17 (m, 2H), 3.92 (t, J = 9.2 Hz, 1H), 3.81 – 3.68 (m, 2H), 3.44 – 3.32 (m, 2H), 3.22 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.1, 165.7 (2xC), 165.2, 138.9, 138.3, 138.2, 133.7, 133.4 (2xC), 133.2, 130.1-127.6 (aromatic region), 102.1, 98.0, 82.0, 79.9, 77.6, 75.6, 74.8, 73.4, 71.7, 71.5, 69.8, 69.7, 68.8, 68.2, 62.0, 55.1.

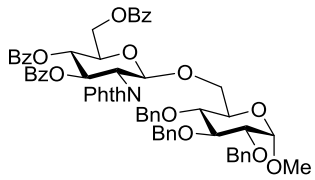


Methyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1,6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (134): General procedure with CSA (0.2 equiv.) and reaction time of 2 hours. Purification by column chromatography (3% acetone in toluene) resulting in 76% yield. **^{1}H NMR**[229] (400 MHz, $CDCl_3$) δ 8.12 – 7.99 (m, 4H), 7.94 – 7.87 (m, 2H), 7.85 – 7.79 (m, 2H), 7.65 – 7.13 (m, 27H), 6.07 (t, J = 10.1 Hz, 1H), 5.88 (dd, J = 10.1, 3.3 Hz, 1H), 5.73 (dd, J = 3.3, 1.8 Hz, 1H), 5.16 (d, J = 1.7 Hz, 1H), 5.02 (d, J = 11.1 Hz, 1H), 5.00 (d, J = 11.1 Hz, 1H), 4.84 (d, J = 11.0 Hz, 1H), 4.80 (d, J = 12.0 Hz, 1H), 4.71 – 4.60 (m, 4H), 4.41 (ddd, J = 9.9, 4.3, 2.3 Hz, 1H), 4.34 (dd, J = 12.0, 4.4 Hz, 1H), 4.04 (t, J = 9.3 Hz, 1H), 3.94 (dd, J = 10.9, 5.1 Hz, 1H), 3.86 (dd, J = 10.1, 5.1 Hz, 1H), 3.81 (dd, J = 11.0, 1.5 Hz, 1H), 3.58 (dd, J = 9.6, 3.6 Hz, 1H), 3.56 – 3.49 (m, 1H), 3.45 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.2, 165.5 (2xC), 165.4, 138.8, 138.3 (2xC), 133.5, 133.3, 133.2, 130.0-127.7 (aromatic region), 98.0, 97.9, 82.2, 80.3, 77.8, 75.8, 75.1, 73.6, 70.4, 70.1, 70.0, 69.0, 67.0, 66.8, 62.8, 55.3.



Methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1,6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (135): General procedure with CSA (0.2 equiv.) and a reaction time of 2 hours. Purification by column chromatography (3% acetone in toluene) resulting in the product in 56% yield. **^{1}H NMR**[222] (400 MHz, $CDCl_3$) δ 7.38 – 7.23 (m, 15H), 5.17 (t, J = 9.4 Hz, 1H), 5.11 – 4.95 (m, 3H), 4.86 (d, J = 10.9 Hz, 1H), 4.82 – 4.76 (m, 2H), 4.65 (d, J

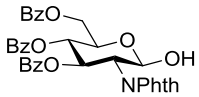
= 12.1 Hz, 1H), 4.58 – 4.50 (m, 3H), 4.23 (dd, J = 12.3, 4.6 Hz, 1H), 4.11 (d, J = 12.2 Hz, 1H), 4.06 (d, J = 10.5 Hz, 1H), 3.97 (t, J = 9.2 Hz, 1H), 3.76 (dd, J = 10.0, 4.1 Hz, 1H), 3.72 – 3.62 (m, 2H), 3.51 (dd, J = 9.6, 3.5 Hz, 1H), 3.42 (t, J = 9.4 Hz, 1H), 3.36 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.95 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 170.7, 170.4, 169.4, 169.1, 138.7, 138.1 (2xC), 128.6-127.6 (aromatic region), 100.7, 98.1, 82.0, 79.8, 77.7, 75.8, 75.0, 73.4, 73.0, 71.8, 71.3, 69.7, 68.4, 68.2, 62.0, 55.2, 20.7 (2xC), 20.6 (2xC).



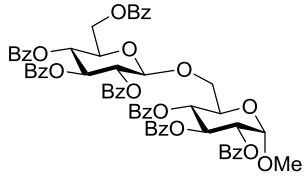
Methyl 3,4,6-tri-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1,6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (136): General Procedure with CSA (0.1 equiv.) and 1 hour reaction time. Purification by column chromatography (1:3:3 EtOAc/Hexane/ Toluene) resulting in 77% yield. **^{1}H NMR**[192] (400 MHz, $CDCl_3$) δ 8.00 (dd, J = 8.3, 1.2 Hz, 2H), 7.89 (dd, J = 8.3, 1.2 Hz, 2H), 7.82 – 7.71 (m, 2H), 7.63 – 7.16 (m, 26H), 7.02 (dd, J = 6.6, 2.9 Hz, 2H), 6.28 (dd, J = 10.7, 9.3 Hz, 1H), 5.69 (t, J = 9.6 Hz, 1H), 5.64 (d, J = 8.5 Hz, 1H), 4.85 (d, J = 10.9 Hz, 1H), 4.73 – 4.52 (m, 6H), 4.43 – 4.38 (m, 2H), 4.30 – 4.21 (m, 1H), 4.15 – 4.11 (m, 2H), 3.84 (t, J = 9.3 Hz, 1H), 3.72 (dd, J = 10.3, 4.7 Hz, 1H), 3.68 (dd, J = 10.3, 3.8 Hz, 1H), 3.39 (dd, J = 9.7, 3.5 Hz, 1H), 3.26 (t, J = 9.4 Hz, 1H), 3.15 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 167.0 (2xC), 166.3, 165.8, 165.3, 138.8, 138.6, 137.9, 134.2 (2xC), 133.5, 133.4, 133.2, 130.0-123.6 (aromatic region), 98.7, 98.0, 82.0, 79.8, 77.8, 75.8, 74.9, 73.5, 72.3, 71.2, 70.5, 69.3, 68.9, 63.5, 55.1, 54.9.

Phenyl 3,4,6-tri-*O*-benzoyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (140):[181] Benzoyl chloride (29.89 mmol) was added dropwise over 20 min to a mixture of phenyl 2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (4.98 mmol) in pyridine (20 ml) at -40°C. Stirring was continued for 5 hours, then the solution was allowed to attain room temperature, then followed by addition of MeOH (30 ml). The mixture was concentrated *in vacuo* and the residue was dissolved in CH_2Cl_2 (100 ml) and washed with 2 M HCl, aq. sat. $NaHCO_3$ and water. The organic layer was dried, filtered and concentrated *in vacuo*.

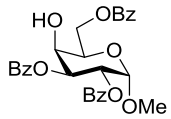
The residue was purified by column chromatography (1:4:4 EtOAc/ Hexane/Toluene) resulting in 99% yield. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.06 – 8.02 (m, 2H, Ar), 7.90 (d, *J* = 7.3 Hz, 2H, Ar), 7.77 – 7.68 (m, 3H, Ar), 7.60 (t, *J* = 7.4 Hz, 1H, Ar), 7.50 – 7.39 (m, 4H, Ar), 7.33 (t, *J* = 7.8 Hz, 2H, Ar), 7.28 – 7.22 (m, 4H, Ar), 7.16 (m, 2H, Ar), 6.31 (t, *J* = 9.8 Hz, 1H, 3-H), 5.92 (d, *J* = 10.5 Hz, 1H, 1-H), 5.66 (t, *J* = 9.7 Hz, 1H, 4-H), 4.68 (dd, *J* = 12.1, 2.6 Hz, 1H, 6a-H), 4.62 (t, *J* = 10.4 Hz, 1H, 2-H), 4.50 (dd, *J* = 12.1, 5.8 Hz, 1H, 6b-H), 4.33 – 4.27 (m, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 168.1 (C=O), 167.1 (C=O), 166.2 (C=O), 165.8 (C=O), 165.3 (C=O), 134.4 (Ar, *ipso*), 133.5 (Ar, *ipso*), 133.3 (Ar, *ipso*), 133.2 (2xC, Ar, *ipso*), 129.9–128.3 (aromatic region), 123.9 (Ar), 83.5 (C-1), 76.4 (C-5), 72.1 (C-3), 70.0 (C-4), 63.3 (C-6), 53.9 (C-2). **HRMS** (MALDI) calcd for $C_{41}H_{31}NO_9S$ $[M+Na]^+$ m/z 736.1612, found 736.1630.



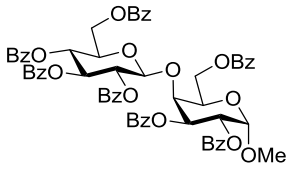
3,4,6-Tri-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranose (141):[191] NBS (3.73 mmol) was added at room temperature to a solution of phenyl 3,4,6-tri-*O*-benzoyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (1.87 mmol) in 9:1 acetone-water (30 ml). The reaction mixture turned orange within 5 minutes. Stirring was continued until the reaction became transparent again after 1 hour. However starting material was still present, and hence more NBS was added until full conversion. The solvent was evaporated and the residue was dissolved in EtOAc, and the organic layer was washed with aq. sat. $NaHCO_3$ and water, then dried and evaporated. The product was isolated by column chromatography (1:3:3 EtOAc/Hexane/Toluene) resulting in 82% yield. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.07 – 8.03 (m, 2H), 7.91 – 7.65 (m, 5H, Ar), 7.58 – 7.13 (m, 8H, Ar), 6.34 (dd, *J* = 10.8, 9.3 Hz, 1H, 3-H), 5.85 (d, *J* = 8.1 Hz, 1H, 1-H), 5.76 – 5.70 (m, 1H, 4-H), 4.66 (dd, *J* = 12.2, 2.9 Hz, 1H, 6a-H), 4.57 – 4.46 (m, 2H, 2-H, 6b-H), 4.32 (ddd, *J* = 10.1, 4.9, 2.9 Hz, 1H, 5-H), 3.42 (bs, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.4 (C=O), 165.8 (C=O), 165.4 (C=O), 134.4 (Ar), 133.6 (Ar), 133.4 (Ar), 133.3 (Ar), 130.0-128.4 (aromatic region), 123.8 (Ar), 93.0 (C-1), 72.5 (C-5), 71.0 (C-3), 70.2 (C-4), 63.2 (C-6), 56.5 (C-2). **HRMS** (MALDI) calcd for $C_{35}H_{27}NO_{10}$ $[M+Na]^+$ m/z 644.1527, found 644.1543.



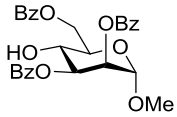
Methyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1,6)-2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside (144): General Procedure with CSA (0.2 equiv.) and 3 hours reaction time. Purification by column chromatography (4:4:1 Toluene/Hexane/EtOAc) resulting in 75% yield. **^{1}H NMR**[230] (400 MHz, $CDCl_3$) δ 8.03 – 7.78 (m, 15H), 7.56 – 7.23 (m, 20H), 6.09 (t, J = 9.8 Hz, 1H), 5.94 (t, J = 9.7 Hz, 1H), 5.68 (t, J = 9.7 Hz, 1H), 5.58 (dd, J = 9.8, 7.9 Hz, 1H), 5.34 (t, J = 9.9 Hz, 1H), 5.11 (dd, J = 10.2, 3.6 Hz, 1H), 5.00 (d, J = 7.9 Hz, 1H), 4.96 (d, J = 3.6 Hz, 1H), 4.63 (dd, J = 12.2, 3.1 Hz, 1H), 4.46 (dd, J = 12.2, 5.1 Hz, 1H), 4.27 – 4.21 (m, 1H), 4.19 – 4.10 (m, 2H), 3.81 (dd, J = 11.3, 7.7 Hz, 1H), 3.12 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.2, 165.9, 165.8, 165.8, 165.5, 165.3 (2xC), 133.6, 133.4, 133.3, 133.2, 133.1, 130.0-128.3 (aromatic region), 101.9, 96.6, 72.9, 72.4, 72.1, 72.0, 70.4, 69.8, 69.7, 69.1, 68.9, 63.1, 55.2.



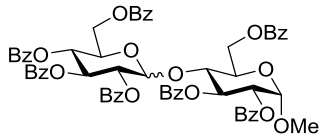
Methyl 2,3,6-tri-O-benzoyl-α-D-galactopyranoside (145):[196] Benzoyl chloride (51.0 mmol) was added dropwise over 45 min to a mixture of methyl α-D-galactopyrano-side (15.5 mmol) in pyridine (30 ml) at -40°C. The reaction mixture was stirred overnight at room temperature. Water (15 ml) was added to the mixture, which was stirred for additional 15 min. Pyridine was removed *in vacuo* and the residue was dissolved in CH_2Cl_2 (50 ml). The organic layer was washed with 2 M HCl, sat. aq. $NaHCO_3$ and brine. The organic layer was dried, filtered and concentrated on silica. The mixture was purified by DCVC (0-80% EtOAc in Hexane) resulting in a white solid in 33% yield. **^{1}H NMR**[231] (400 MHz, $CDCl_3$) δ 8.07 – 7.96 (m, 6H), 7.61 – 7.34 (m, 9H), 5.76 (dd, J = 10.7, 3.0 Hz, 1H), 5.69 (dd, J = 10.7, 3.5 Hz, 1H), 5.22 (d, J = 3.5 Hz, 1H), 4.68 (dd, J = 11.5, 5.9 Hz, 1H), 4.57 (dd, J = 11.4, 6.8 Hz, 1H), 4.41 (d, J = 2.5 Hz, 1H), 4.36 (t, J = 6.4 Hz, 1H), 3.45 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.6, 166.2, 165.9, 133.6, 133.4 (2xC), 130.0-128.5 (aromatic region), 97.7, 70.9, 69.0, 68.3, 67.8, 63.5, 55.7.



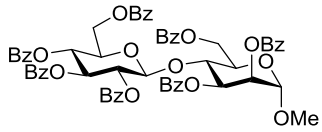
Methyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1,4)-2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside (146): General Procedure with TfOH (0.3 equiv.) and reaction time of 24 hours. Purification by DCVC (0-70% EtOAc in Hexane) resulting in a white solid in 81% yield. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.05 – 7.92 (m, 8H, Ar), 7.88 – 7.77 (m, 6H, Ar), 7.61 – 7.24 (m, 19H, Ar), 7.13 (t, J = 7.8 Hz, 2H, Ar), 5.87 – 5.81 (m, 2H, 3-H, 3'-H), 5.71 – 5.62 (m, 2H, 2'-H, 4'-H), 5.26 (dd, J = 10.7, 3.6 Hz, 1H, 2-H), 5.20 (d, J = 3.6 Hz, 1H, 1-H), 5.09 (d, J = 7.8 Hz, 1H, 1'-H), 4.73 (dd, J = 11.7, 4.7 Hz, 1H, 6a-H), 4.58 – 4.52 (m, 2H, 4-H, 6b-H), 4.47 (dd, J = 12.2, 3.3 Hz, 1H, 6a'-H), 4.42 – 4.34 (m, 2H, 6b'-H), 3.96 (dt, J = 9.8, 3.9 Hz, 1H, 5'-H), 3.34 (s, 3H, OMe). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.1 (C=O), 166.1 (C=O), 166.0 (2xC, C=O), 165.5 (C=O), 165.3 (C=O), 165.2 (C=O), 133.8 (Ar), 133.5 (Ar), 133.4 (Ar), 133.2 (Ar), 133.1 (2xC, Ar), 133.0 (Ar), 130.1-128.2 (aromatic region), 101.3 (C-1'), 97.3 (C-1), 74.9 (C-4), 72.8 (C-3'), 72.4 (C-5'), 72.2 (C-2'), 70.5 (C-3), 69.7 (C-2), 69.5 (C-4'), 67.9 (C-5), 64.2 (C-6), 62.6 (C-6'), 55.5 (OMe). **HRMS** (MALDI) calcd for $C_{62}H_{52}O_{18}$ $[M+Na]^+$ m/z 1107.3046, found 1107.3069.



Methyl 2,3,6-tri-*O*-benzoyl-α-D-mannopyranoside (148):[196] Benzoyl chloride (51.0 mmol) was added dropwise over 30 min to D-mannose (15.5 mmol) in pyridine (30 ml) at -78 °C. The reaction mixture was stirred overnight at room temperature. Water (15 ml) was added and the mixture was stirred for an additional 30 min, then all solvent was removed *in vacuo*. The residue was dissolved in CH_2Cl_2 (70 ml) and washed with 2 M HCl, sat. aq. $NaHCO_3$ and water. The organic phase was dried, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (5% acetone in toluene) resulting in the product in 39% yield. **^{1}H NMR**[232] (400 MHz, $CDCl_3$) δ 8.13 (d, J = 7.6 Hz, 2H), 7.99 (d, J = 7.7 Hz, 2H), 7.92 (d, J = 7.7 Hz, 2H), 7.64 – 7.53 (m, 2H), 7.53 – 7.44 (m, 3H), 7.33 (t, J = 7.5 Hz, 4H), 5.66 – 5.61 (m, 2H), 4.94 (s, 1H), 4.89 (dd, J = 12.0, 2.7 Hz, 1H), 4.67 (d, J = 12.0 Hz, 1H), 4.31 (t, J = 9.5 Hz, 1H), 4.11 (d, J = 9.6 Hz, 1H), 3.50 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 167.0, 166.8, 165.5, 133.5, 133.4, 133.3, 130.0-128.5 (aromatic region), 98.8, 72.7, 71.3, 70.6, 66.4, 63.5, 55.5.

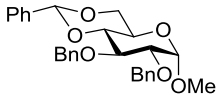


Methyl 2,3,4,6-tetra-*O*-benzoyl-D-glucopyranosyl-(1,4)-2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside (149): General Procedure with TfOH (0.2 equiv.) and reaction time of 6 hours. Purification by DCVC (0-18% EtOAc in Toluene/Hexane) resulting in both anomers in the ratio 1:2 (α/β) in 80% yield. **α-form**: **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.14 – 8.10 (m, 2H, Ar), 8.03 – 7.99 (m, 2H, Ar), 7.90 – 7.86 (m , 4H, Ar), 7.77 – 7.73 (m, 4H, Ar), 7.70 – 7.66 (m, 2H, Ar), 7.62 – 7.17 (m, 21H, Ar), 6.10 (t, J = 9.5 Hz, 2H, 3-H, 3'-H), 5.79 (d, J = 3.8 Hz, 1H, 1'-H), 5.68 (t, J = 9.8 Hz, 1H, 4'-H), 5.28 (dd, J = 10.5, 3.9 Hz, 1H, 2'-H), 5.16 (d, J = 3.5 Hz, 1H, 1-H), 5.09 (dd, J = 10.2, 3.5 Hz, 1H, 2-H), 4.90 (dd, J = 12.1, 1.8 Hz, 1H, 6a-H), 4.77 (dd, J = 12.1, 4.1 Hz, 1H, 6b-H), 4.52 – 4.42 (m, 3H, 4-H, 5'-H, 6a'-H), 4.39 – 4.35 (m, 1H, 5-H), 4.31 (dd, J = 12.2, 3.5 Hz, 1H, 6b-H), 3.47 (s, 3H, OMe). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.3 (C=O), 166.1 (C=O), 166.0 (C=O), 165.8 (C=O), 165.6 (C=O), 165.2 (C=O), 165.0 (C=O), 133.6 (Ar), 133.5 (Ar), 133.4 (2xC, Ar), 133.2 (3xC, Ar), 130.2-128.2 (aromatic region), 96.9 (C-1), 96.7 (C-'1), 73.7 (C-4), 72.6 (C-2), 72.5 (C-3), 71.0 (C-2'), 70.0 (C-3'), 69.3 (C-4'), 69.2 (C-5'), 68.4 (C-5), 63.6 (C-6), 62.6 (C-6'), 55.7 (OMe). **HRMS** (MALDI) calcd for $C_{62}H_{52}O_{18}$ $[M+Na]^+$ m/z 1107.3046, found 1107.3068. **β-form**: **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.06 – 7.94 (m, 10H, Ar), 7.81– 7.74 (m, 4H, Ar), 7.59 – 7.14 (m, 21H, Ar), 6.09 (t, J = 9.5 Hz, 1H, 3-H), 5.77 (t, J = 9.6 Hz, 1H, 3'-H), 5.55 (dd, J = 9.6, 8.0 Hz, 1H, 2'-H), 5.43 (t, J = 9.5 Hz, 1H, 4'-H), 5.18 (dd, J = 10.2, 3.6 Hz, 1H, 2-H), 5.11 (d, J = 3.6 Hz, 1H, 1-H), 5.00 (d, J = 7.9 Hz, 1H, 1'-H), 4.61 (dd, J = 12.0, 1.1 Hz, 1H, 6a-H), 4.51 (dd, J = 12.1, 4.3 Hz, 1H, 6b-H), 4.21 (t, J = 9.5 Hz, 1H, 4-H), 4.11 (m, 2H, 5-H, 6a'-H), 3.86 (m, 2H, 5-'H, 6b'-H), 3.36 (s, 3H, OMe). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.1 (C=O), 166.0 (C=O), 165.8 (2xC, C=O), 165.4 (C=O), 165.1 (C=O), 164.9 (C=O), 133.4 (3xC), 133.32 (3xC, Ar), 133.2, 130.0-128.4 (aromatic region), 101.1 (C-1'), 96.9 (C-1), 76.8 (C-4), 73.1 (C-3'), 72.5 (C-5'), 72.2 (C-2'), 72.1 (C-2), 70.3 (C-3), 69.5 (C-4'), 68.5 (C-5), 62.7 (C-6'), 62.5 (C-6), 55.6 (OMe). **HRMS** (MALDI) calcd for $C_{62}H_{52}O_{18}$ $[M+Na]^+$ m/z 1107.3046, found 1107.3069.

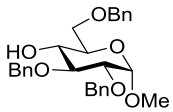


Methyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1,4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranoside (150): General Procedure with TfOH (0.2 equiv.) and reaction time of 2 hours. Purification by column chromatography (1:4:4 EtOAc/Hexane/Toluene) resulting in 80% yield. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.01 – 7.71 (m, 14H, Ar), 7.61 – 7.17 (m, 21H, Ar), 5.87 (dd, J = 9.5, 3.5 Hz, 1H, 3-H), 5.79 (t, J = 9.7 Hz, 1H, 3'-H), 5.62

(dd, J = 3.4, 1.9 Hz, 1H, 2-H), 5.56 – 5.51 (m, 2H, 2'-H, 4'-H), 5.07 (d, J = 7.9 Hz, 1H, 1'-H), 4.83 (d, J = 1.8 Hz, 1H, 1-H), 4.69 (dd, J = 11.9, 1.7 Hz, 1H, 6a-H), 4.56 – 4.47 (m, 2H, 4-H, 6b-H), 4.13 – 4.06 (m, 3H, 5-H, 6'-H), 3.78 (dt, J = 9.7, 3.7 Hz, 1H, 5'-H), 3.41 (s, 3H, OMe). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 165.9 (3xC, C=O), 165.4 (C=O), 165.1 (C=O), 165.0 (2xC, C=O), 133.5, 133.4 (3xC), 133.3, 133.1 (2xC), 130.0-128.3 (aromatic region), 101.3 (C-1'), 98.6 (C-1), 74.5 (C-4), 72.9 (C-3'), 72.3 (C-5'), 72.2 (C-2'), 70.5 (C-2), 70.2 (C-3), 69.44 (C-4'), 69.4 (C-5), 62.8 (C-6'), 62.5 (C-6), 55.5 (OMe). **HRMS** (MALDI) calcd for $C_{62}H_{52}O_{18}$ $[M+Na]^+$ m/z 1107.3046, found 1107.3079.

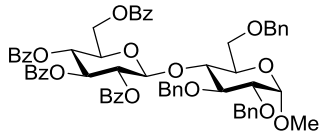


Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (151):[233] Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (21.9 mmol) and NaH (83 mmol, 50% in mineral oil) in dry DMF (100 ml) were stirred for 30 min and then cooled to 0°C under an argon atmosphere. Benzyl bromide (66 mmol) and 0.54 g Bu_4NI (1.5 mmol) were added and the solution stirred overnight. The mixture was quenched with MeOH (5 ml) and diluted with CH_2Cl_2 (80 ml) and washed with water. The organic layer was dried and concentrated. The residue was crystallized from ethanol yielding a white solid in 77% yield. **^{1}H NMR**[234] (400 MHz, $CDCl_3$) δ 7.51 – 7.47 (m 2H), 7.41 – 7.25 (m, 13H), 5.55 (s, 1H), 4.93 – 4.82 (m, 3H), 4.70 (d, J = 12.2 Hz, 1H), 4.60 (d, J = 3.7 Hz, 1H), 4.27 (dd, J = 10.1, 4.7 Hz, 1H), 4.05 (t, J = 9.3 Hz, 1H), 3.83 (td, J = 9.9, 4.7 Hz, 1H), 3.71 (t, J = 10.2 Hz, 1H), 3.61 (t, J = 9.4 Hz, 1H), 3.56 (dd, J = 9.3, 3.7 Hz, 1H), 3.41 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 138.9, 138.3, 137.6, 129.1-127.7 (aromatic region), 126.2, 101.4, 99.4, 82.3, 79.3, 78.8, 75.5, 74.0, 69.2, 62.5, 55.5.

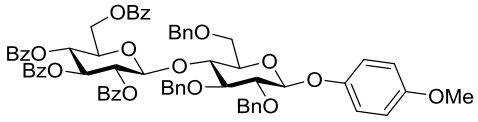


Methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (152):[235] Methyl 2,3-di-*O*-benzyl 4,6-*O*-benzylidene-α-D-glucopyranoside (10.8 mmol) was dissolved in dry CH_2Cl_2 (20 ml) and cooled at 0°C. Trifluoroacetic anhydride (32.4 mmol) and triethylsilane (54.1 mmol) were added. After 5 min at 0°C trifluoroacetic acid (54.1 mmol) was added drop wise. The reaction mixture was stirred for 2 hours at room temperature, then diluted with EtOAc and washed with aq. sat. $NaHCO_3$ and brine, and then dried and concentrated *in vacuo*. The crude product was purified with column chromatography (hexane/EtOAc 4:1). The product was obtained as colorless oil in 66% yield. **^{1}H NMR**[234] (400 MHz, $CDCl_3$) δ 7.23 –

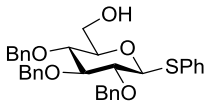
7.18 (m, 15H), 4.95 (d, J = 11.4 Hz, 1H), 4.70 (m, 2H), 4.59 (dd, J = 9.9, 7.9 Hz, 2H), 4.55 – 4.46 (m, 2H), 3.73 (t, J = 9.1 Hz, 1H), 3.68 – 3.61 (m, 3H), 3.55 (td, J = 9.2, 2.1 Hz, 1H), 3.48 (dd, J = 9.5, 3.5 Hz, 1H), 3.33 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 138.9, 138.1 (2xC), 128.7-127.8 (aromatic region), 98.3, 81.6, 79.7, 75.6, 73.7, 73.3, 70.8, 70.0 69.6, 55.4.



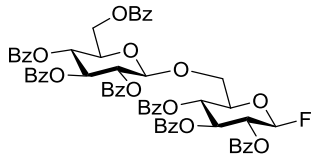
Methyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1,4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (153): General Procedure with CSA (0.2 equiv.) and reaction time of 2-24 hours. Purification by column chromatography (4:4:1 Toluene/Hexane/EtOAc) resulting in yields from 20-40% estimated by NMR.[226] **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.00-7.80 (m, 8H), 7.61 – 7.03 (m, 27H), 5.61 (d, J = 9.5 Hz, 1H), 5.55 (t, J = 9.6 Hz, 1H), 5.47 (dd,J = 9.5 Hz, 8.0, 1H), 5.07 (d, J = 11.2 Hz, 1H), 4.85 – 4.71 (m, 4H), 4.70 – 4.37 (m, 3H), 4.34 (d, J = 12.1 Hz, 1H), 4.31 – 4.22 (m, 1H), 3.97 (t, J = 9.4 Hz, 1H), 3.88 (t, J = 9.2 Hz, 1H), 3.75 – 3.67 (m, 2H), 3.57 – 3.34 (m, 3H), 3.28 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.1, 165.8, 165.1, 164.8, 139.3, 138.4, 137.9, 133.4, 133.3, 133.2, 133.0, 129.9-127.2, 100.4, 98.5, 80.0, 78.8, 77.3, 75.4, 73.64, 73.6, 73.2, 72.3, 71.8, 69.9, 69.5, 67.6, 63.2, 55.4. **Methyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1,4)-2,6-tri-*O*-benzyl-α-D-glucopyranoside:** By-Product **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.18 – 8.07 (m, 2H, Ar), 8.00 – 7.87 (m, 2H, Ar), 7.86 – 7.72 (m, 2H, Ar), 7.59 – 7.17 (m, 22H, Ar), 5.82 (t, J = 9.7 Hz, 1H, H-3'), 5.63 – 5.52 (m, 2H, H-2', H-4'), 4.86 (d, J = 12.4 Hz, 1H, $PhCH_2O$), 4.75 (d, J = 7.9 Hz, 1H, H-1'), 4.75 – 4.69 (m, 1H, H-6b'), 4.61 (d, J = 12.4 Hz, 1H, $PhCH_2O$), 4.55 – 4.48 (m, 1H, H-1, $PhCH_2O$), 4.42 (dd, J = 12.4, 6.8 Hz, 1H, H-6b), 4.37 (d, J = 12.1 Hz, 1H, $PhCH_2O$), 4.15 (d, J = 12.7 Hz, 1H, $PhCH_2O$), 4.12 (m, 1H, H-5'), 4.06 (m, 1H, H-3), 3.72 – 3.57 (m, 2H, H-4, H-5), 3.45 (dd, J = 10.7, 3.0 Hz, 1H, H-6a), 3.40 – 3.31 (m, 2H, H-2, H-6b), 3.26 (s, 3H, OMe). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.3 (C=O), 165.9 (C=O), 165.3 (C=O), 165.0 (PhC=O), 138.8, 138.2, 133.8, 133.7, 133.5, 133.4, 130.2 - 127.8 (aromatic region), 101.4 (C-1'), 98.7 (C-1), 81.5 (C-4), 78.5 (C-2), 73.8 ($PhCH_2O$), 73.3 ($PhCH_2O$), 72.80 (C-3', C-5'), 72.1 (C-3), 71.8 (C-2'), 69.5 (C-4'), 68.5 (C-5), 67.6 (C-6), 63.2 (C-6'), 55.5 (OMe).



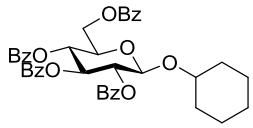
***p*-Methoxyphenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1,4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (155):** General Procedure with CSA (0.2 equiv.) and reaction time of 5 hours. Purification by column chromatography (0-2% acetone in toluene) resulting in 31% yield. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.98 (d, J = 7.3 Hz, 2H), 7.94 – 7.86 (m, 4H), 7.81 (d, J = 7.4 Hz, 2H), 7.56 – 7.16 (m, 29H), 6.98 – 6.90 (m, 1H), 6.79 – 6.71 (m, 1H), 5.72 (t, J = 9.6 Hz, 1H, 3'-H), 5.59 (t, J = 9.7 Hz, 1H, 4'-H), 5.54 – 5.48 (m, 1H, 2'-H), 5.10 (d, J = 11.3 Hz, 1H, $PhCH_2O$), 4.98 (d, J = 8.0 Hz, 1H), 4.91 – 4.74 (m, 4H, $PhCH_2O$, 1-H), 4.67 (d, J = 12.0, 1H, $PhCH_2O$), 4.43 (dd, J = 12.1, 3.1 Hz, 1H, 6a'-H), 4.38 (d, J = 12.0 Hz, 1H, $PhCH_2O$), 4.27 (dd, J = 12.1, 4.9 Hz, 1H, 6b'-H), 4.09 (t, J = 9.2 Hz, 1H, 4-H), 3.83 – 3.58 (m, 8H, OMe, 5'-H, 3-H, 2-H, 6-H), 3.37 (d, J = 7.5 Hz, 1H, 5-H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.1 (C=O), 165.9 (C=O), 165.2 (C=O), 165.0 (C=O), 151.6, 139.0, 138.3, 138.1, 133.6, 133.5, 133.3, 133.1, 129.9-127.4 (aromatic region), 123.0, 117.3, 112.4, 102.6 (C-1), 100.6 (C-1'), 82.6 (C-3), 81.5 (C-2), 75.4 ($PhCH_2O$), 75.2 ($PhCH_2O$), 74.6 (C-5), 73.6 ($PhCH_2O$), 73.2 (C-3'), 72.4 (C-2'), 72.1 (C-5'), 69.8 (C-4'), 67.8 (C-6), 63.1 (C-6'), 56.8 (OMe). **HRMS** (MALDI) calcd for $C_{68}H_{62}O_{16}$ $[M+Na]^+$ m/z 1157.3930, found 1157.3956.



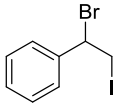
Phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (157):[236] Phenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (2.77 mmol) was treated with BH_3·THF (7.63 mmol, 1 M THF solution). After stirring for 10 min $Cu(OTf)_2$ (0.14 mmol) was added at 0 °C. The reaction was stirred for 4 days, and then quenched by sequential addition of Et_3N and MeOH. The resulting mixture was co-evaporated with MeOH and the residue purified by column chromatography (1:4:4 EtOAc/Hexane/Toluene) resulting in a white solid in 77% yield. **^{1}H NMR**[237] (400 MHz, $CDCl_3$) δ 7.53 – 7.50 (m, 2H), 7.41 – 4.22 (m, 14H), 9.95 – 4.84 (m, 4H), 4.79 – 4.63 (m, 3H), 3.87 (dd, J = 12.0, 2.7 Hz, 1H), 3.73 (t, J = 8.9 Hz, 1H), 3.69 (dd, J = 11.4, 4.2 Hz, 1H), 3.58 (t, J = 9.4 Hz, 1H), 3.49 (dd, J = 9.7, 8.9 Hz, 1H), 3.39 (ddd, J = 9.7, 4.9, 2.7 Hz, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 138.3, 137.9, 131.9, 129.1-127.7 (aromatic region), 87.6, 86.6, 81.1, 79.3, 77.6, 75.9, 75.6, 75.1, 62.2.



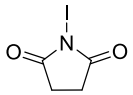
2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1,6)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl fluoride (160): General Procedure with CSA (0.2 equiv.) and reaction time of 1.5 hours. Purification by DCVC (0-6% acetone in toluene) resulting in 62% yield. **^{1}H NMR**[238] (400 MHz, $CDCl_3$) δ 8.05 – 7.81 (m, 14H), 7.53 – 7.26 (m, 21H), 5.91 (t, J = 9.7 Hz, 1H, H-3'), 5.74 (t, J = 8.5 Hz, 1H, H-3), 5.65 (t, J = 9.7 Hz, 1H, H-4'), 5.53 (dd, J = 9.5, 8.1 Hz, 1H, H-2'), 5.50 – 5.42 (m, 2H, H-2, H-4), 5.27 (dd, J = 51.5, 5.8 Hz, 1H, H-1), 5.02 (d, J = 7.9 Hz, 1H, H-1'), 4.61 (dd, J = 12.2, 3.0 Hz, 1H, H-6a'), 4.45 (dd, J = 12.2, 4.9 Hz, 1H, H-6b'), 4.18 – 4.11 (m, 3H, H-5, H-5', H-6a), 3.95 (dd, J = 12.3, 8.2 Hz, 1H, H-6b). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.3, 165.9, 165.5, 165.4, 165.3 (2xC), 165.0, 133.8, 133.7 (2xC), 133.6 (2xC), 133.4, 133.3, 130.1 – 128.4, 106.6 (d, J = 220.2 Hz, C-1), 102.1 (C-1'), 74.5 (d, J = 3.6 Hz, C-5), 72.9 (C-3'), 72.5 (C-5'), 71.9 (C-2'), 71.6 (d, J = 23.4 Hz, C-2), 71.4 (d, J = 13.9 Hz, C-3), 69.7 (C-4'), 69.2 (C-6), 68.6 (C-4), 63.0 (C-6').



Cyclohexyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside (161):[15] Cyclohexanol (2.0 mmol) and 2,3,4,6-tetra-O-benzoyl-α-D-glucopyranosyl bromide (1.0 mmol) were stirred in CH_2Cl_2 (4 ml) at room temperature under a nitrogen atmosphere, followed by addition of silver oxide (2.0 mmol). The reaction was stirred overnight, then diluted, filtered through Celite™ and solvent was removed *in vacuo*. The crude product was purified by flash column chromatography (1:4:4 Acetone/Hexane/Toluene) resulting in 84% yield. **^{1}H NMR**[225] (400 MHz, $CDCl_3$) δ 8.05 – 8.00 (m, 2H), 8.00 – 7.94 (m, 2H), 7.91 (dd, J = 8.2, 1.1 Hz, 2H), 7.88 – 7.82 (m, 2H), 7.58 – 7.22 (m, 12H), 5.91 (t, J = 9.6 Hz, 1H), 5.65 (t, J = 9.7 Hz, 1H), 5.52 (dd, J = 9.8, 7.9 Hz, 1H), 4.95 (d, J = 7.9 Hz, 1H), 4.63 (dd, J = 12.0, 3.4 Hz, 1H), 4.53 (dd, J = 12.0, 5.8 Hz, 1H), 4.16 (ddd, J = 9.5, 5.8, 3.4 Hz, 1H), 3.72 – 3.61 (m, 1H), 1.92 – 1.86 (m, 1H), 1.80 – 1.53 (m, 3H), 1.52 – 1.37 (m, 2H), 1.33 – 1.03 (m, 4H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.3, 166.0, 165.4, 165.2, 133.5, 133.3, 133.2 (2xC), 130.2 - 128.4 (aromatic region), 100.0, 78.6, 73.2, 72.2 (2xC), 70.2, 63.6, 33.4, 31.8, 25.5, 23.8, 23.7.



1-Bromo-2-iodo-1-phenylethane (162): To a solution of styrene (0.96 mmol) in dry $CDCl_3$ (1 ml) was added IBr (1.06 mmol) in dry $CDCl_3$ (1.0 ml) dropwise at room temperature under nitrogen atmosphere. ^{1}H and ^{13}C NMR were obtained after 2 and 24 hours. After 2 hours styrene is entirely consumed, which is evident by the disappearance of the carbon shift at 113.8 ppm belonging to the terminal alkene of styrene. The spectra obtained **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.43 – 7.29 (m, 5H), 5.22 (dd, J = 11.6, 4.9 Hz, 1H), 4.03 (dd, J = 9.9, 4.9 Hz, 1H), 3.97 (dd, J = 11.5 Hz, 9.9 Hz 1H) and **^{13}C NMR**[215] (101 MHz, $CDCl_3$) δ 139.6, 129.3, 129.0, 127.7, 51.1, 9.5. It was not possible isolate the product by evaporation of the solvent.



***N*-iodosuccinimide:**[208] NCS (10.04 mmol) and NaI (10.04 mmol) were dissolved separately in dry acetone (25 ml), then followed by addition of the NCS solution to the solution of NaI and the resulting mixture turned orange at once. The resulting mixture was stirred for 2 hours in which precipitation occurred. The reaction was diluted with dry acetone (50 ml) and the precipitate was filtered off and the solvent evaporated. NIS was purified by recrystallizing the residue from CCl_4-dioxane[239] resulting 44% yield. **^{1}H NMR**[240] (400 MHz, DMSO) δ 2.71 (s, 1H). **^{13}C NMR** (101 MHz, DMSO) δ 180.6, 29.5.

Anomerization Procedure: Cyclohexyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside (0.22 mmol) was dissolved in $CDCl_3$ (1 ml) at room temperature under a nitrogen atmosphere. The mixture was shielded from light with silver foil. Anomerization reagent (NIS (2.0 equiv.), IBr (2.0 equiv.), HBr (3.0 equiv.) or HBr (0.1 equiv.)) was added to the mixture. The potential anomerization was monitored by ^{13}C NMR.

Procedure for quenching with styrene: Cyclohexanol (0.5 mmol) and 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide (0.25 mmol) were stirred in dry $CDCl_3$ (1 ml) at room temperature under nitrogen, then NIS (0.37 mmol) was added. After 5 hours full conversion of the starting material was observed, and the reaction was quenched with styrene and stirred for an additional 1 hour. ^{13}C NMR was obtained from the crude. Both β/α cyclohexyl 2,3,4,6-tetra-*O*-benzoyl-D-glucopyranoside (100.0 ppm and 94.8 ppm) were identified along with bromo-2-iodo-1-phenylethane (51.12 ppm and 9.53 ppm).

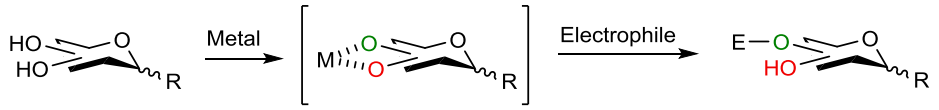
3 Metal-mediated Regioselective Coupling of Unprotected Carbohydrates

Glycosylation is the key reaction in the synthesis of oligosaccharides. This part of the thesis is concerned with investigations regarding the regioselective formation of the glycosidic linkage. The complexity of a glycosylation reaction can be illustrated with the coupling of two glucose molecules, which can give rise to five regioselective products by itself. For this reason alone, assembly of oligosaccharides still represents a challenge, and creates the need for protecting groups to selectively block hydroxyl groups to avoid undesired products. Placing and removing protecting groups creates a number of additional steps in every strategy to synthesize oligosaccharide targets. Therefore, an approach to avoide or decrease the number of protecting groups is of the outmost interest.
In nature, the enzymes recognize substrates and manipulate them with a minimum of interactions. These substrates will undergo site selective modifications, which in case of carbohydrates will involve both regio- and stereoselective aspects. Being able to utilize reagents to create this type of site selective behavior with unprotected carbohydrate in chemical synthesis would be highly desireable.

3.1 Aim of the Project

We anticipate that it is possible to reduce the number of steps in the chemical synthesis of carbohydrates by employing metal complexes to mediate the regioselective glycosylation of unprotected glycosyl acceptors. The objective in this part of the thesis is to investigate possible new strategies for the regioselective formation of glycosidic linkages between carbohydrate moieties by employing metals, and hereby avoiding the need for protecting groups. Complexation of the metal with a diol belonging to a pyranose substrate is likely to result in stronger ionization of one

hydroxyl group over the other. The ionization should increase nucleophilicity and enable the hydroxyl group to react preferentially with an electrophile, in contrast to the remaining hydroxyl groups as shown in Scheme 68.



Scheme 68 Illustration of complexation and regioselective glycosylation with metal.

Various metal complexes in combination with unprotected pyranosides are investigated for the ability to differentiate between the hydroxyl groups of the acceptor and influencing regioselectivity of the glycosidic bond formation. The investigations presented here are to some extend a continuation of recent work of the group[166,168,175] and an exploration of other metal complexes.

The work conducted earlier in the group[166,168,175] employed tin and boron reagents to direct the glycosylation of unprotected carbohydrate acceptors as described in the introduction. Most commonly thioglycopyranosides have been explored as acceptors, since they also are useful glycosyl donors. In line with the aforementioned work of the group, the investigations presented in here are conducted by utilizing fully unprotected pyranosides, which encompass gluco-, galacto- and mannopyranosides. In a straightforward setup, the glycosyl acceptors were treated with a given metal complex followed by regioselective coupling with a highly disarmed glycosyl donor in a Koenigs-Knorr type reaction.

The metal-directed approach will likely not provide access to all the conceivable glycosidic linkages, since the inherent selectivity is prone to favour some linkages over others. However, the metal-directed regioselective glycosylation could give rapid access to a range of building blocks for synthesis of larger oligosaccharides. Therefore, a metal-directed glycosylation approach could be an important addition to the collection of tools in carbohydrate synthesis.

3.2 Glycosyl Acceptors for Glycosylation

The synthesis of the disarmed glycosyl donors employed in this projects were described in the previous project presented in this thesis. In this section, the applied glycosyl acceptors will be discussed.

The glycosyl acceptors employed in the investigations in this chapter are all fully unprotected containing different configuration of the hydroxyl groups and the anomeric substituents.

Methyl α-D-glucopyranoside **(69)** and methyl α-D-mannopyranoside **(104)** were commercially available, along with tolyl 1-thio-β-D-glucopyranoside **(166)**. Also, benzyl α-D-mannopyranoside **(163)**[241], phenyl 1-thio-α-D-mannopyranoside **(164)**[241] and phenyl 1-thio-β-D-xylopyranoside **(165)**[242] were readily available.

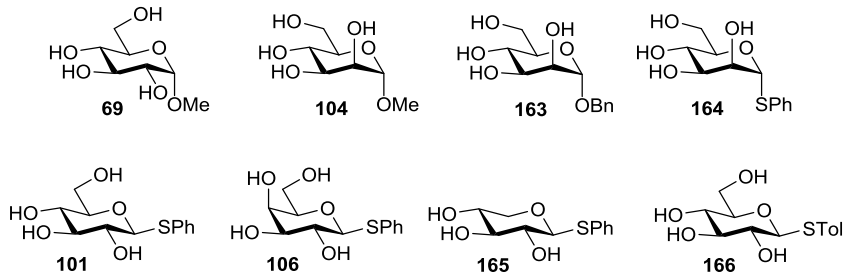


Figure 13 Glycosyl acceptors employed for the investigations.

Phenyl 1-thio-β-D-glucopyranoside **(101)** and phenyl 1-thio-β-D-galactopyranoside **(106)** were both prepared from peracetylated glucopyranose and galactopyranose respectively, by treatment with boron trifluoride etherate and thiophenol. In both cases the resulting crude product was subjected to deacetylation using Zemplen conditions.[243] Phenyl 1-thio-β-D-glucopyranoside **(101)** was obtained in 81% yield from phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside **(167)** and phenyl 1-thio-β-D-galactopyranoside **(106)** in 17% yield overall in two steps from peracetylated D-galactose.

3.3 Copper

The idea of employing copper for regioselective glycosylation originated in the work of Evgeny Evtushenko.[153] Evtushenko utilized copper complexes to facilitate regioselective benzoylation of unprotected glucopyranosides. Regioselective benzoylation of the 2 position in methyl-α-L-rhamnopyranoside was achieved with copper(II) trifluoroacetate, copper(II) perchlorate and copper(II) triflate in the presence of collidine and with little influence of the solvents (*vide supra*).[153] The copper reagent was suggested to form complexes with 1,2-*cis* diols or between methyl acetal and the neighboring hydroxyl group. Otherwise, in the absence of 1,2-*cis* diols, coupling occured at the hydroxyl group possessing the strongest inherent selectivity.[153]

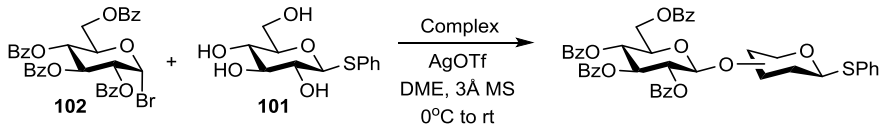
The conditions for the regioselective benzoylation were transferred to a setup for glycosylations, which resembles our prior experience from the tin-mediated glycosylation.[166] The setup for the experiments is shown in Table 7.

2,3,4,6-Tetra-*O*-benzoyl-α-D-glucopyranosyl bromide **(102)** was applied as the glycosyl donor and phenyl 1-thio-β-D-glucopyranoside **(101)** was used as the glycosyl acceptor. Silver triflate was employed with this highly disarmed glycosyl donor for more efficient activation. The acidity of silver(I) triflate was not considered an issue, since metal triflates had been employed for the regioselective manipulation by Evtushenko.[153] Literature indicated that a base was needed to facilitate interaction of the copper complex with the diol, which included NaH[149,150,152], *N,N*-diisopropylethylamine[154,155] (DIPEA) and 2,4,6-collidine[153]. Based on the literature, collidine was chosen for the initial screening.

Different copper complexes were tested in a setup with the aforementioned donor and acceptor, as shown in Table 7. The solubility of the acceptor was a limitation to the setup and therefore an important consideration. However, no indication of great solvent influence on the regioselective manipulation was found in the literature[153] and therefore different solvents

were screened. These solvents included dichloromethane, dimethoxyethane (DME) and THF, since they had been employed previously in connection with manipulation of glycopyranosides and copper complexes.[149,155,244] Especially, DME was considered as a suitable solvent, due to the solubility of the acceptor and the stability in the presence of the promoter.

Table 7 Conditions investigated for regioselective glycosylation with copper.



Entry	Complex	Additive	Time (h)
1	$Cu(OTf)_2$	Collidine	1
2[a,c]	$Cu(OTf)_2$	Collidine, 4Å MS	1
3[b,c]	$Cu(OTf)_2$	Collidine	2
4	$Cu(OTf)_2$		1
5	Copper(II) 2-ethylhexanoate		1
6	Copper(II) 2-ethylhexanoate	Collidine	2
7	$Cu_2(TFA)_4(THF)_2$		2
8	$Cu_2(TFA)_4(THF)_2$	Collidine	1
9	$Cu(TFA)_2$		48
10[d]	$Cu(TFA)_2$		24

Conditions: Acceptor (0.7 mmol), copper complex (0.7 mmol), collidine (0.7 mmol), Donor (1.1 mmol), DME (6 mL/mmol), 3Å MS, AgOTf (1.3 mmol). [a] CH_2Cl_2, [b]THF, [c]rt, [d]Ag_2O

In entry 1, 2 and 3 (Table 7), the solvents were investigated. In each case, phenyl 1-thio-β-D-glucopyranoside **(101)** was stirred with copper(II) triflate and collidine at 0°C for 1 hour, which was followed by the addition of 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide **(102)** and silver(I) triflate. In all cases, the reactions turned grey, hereby indicating the

formation of silver bromide. Furthermore, disappearance of the donor and the acceptor were observed. The hydrolyzed donor and the 1,2-benzoyl migration product were the only compounds isolated. An experiment without the base was conducted (Entry 4, Table 7), but with the same result as before.

Other copper complexes were investigated, including copper(II) 2-ethylhexanoate, which was examined both with and without the base (Entries 5 & 6, Table 7). In both cases the donor and the hydrolysis product of the donor was recovered, along with complex mixtures containing no traces of disaccharide products.

Copper(II) trifluoroacetic acid was considered as an alternative based on the literature.[153] However, this complex was not readily available, and therefore synthesized as the dimeric equivalent $Cu_2(TFA)_4(THF)_2$ using copper(II) methoxide and trifluoroacetic acid (TFA) under inert conditions, which resulted in turquoise crystals in 44% yield.

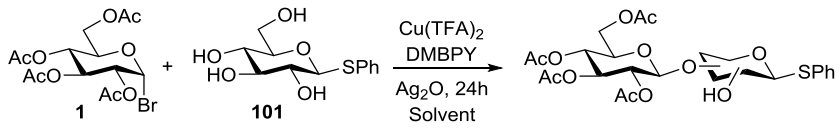
Two experiment were conducted with $Cu_2(TFA)_4(THF)_2$ as above, i.e. one with the base (Entry 8, Table 7) and one without (Entry 7, Table 7). The results were the same as in the previous attempts. The same reaction was done with $Cu(TFA)_2$ from the supplier (Entry 9, Table 7), but transformation to the desired disaccharide product was not observed.

Looking deeper into the literature it was discovered, that Suthers *et al.*[152] recovered the donor, when employing AgOTf as the promoter for glycosyl bromides in regioselective glycosylation with copper complexes. Allen and Miller[154] glycosylated partially unprotected D-glucose with 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide employing a chiral copper complex, base and silver(I) oxide. Therefore, more neutral conditions were considered instead of the highly acidic conditions created by silver(I) triflate. In entry 10 (Table 7), the reaction was activated with silver(I) oxide, but after 24 hours the starting materials were still present.

The more reactive 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **(1)** was employed as the donor in the setup in Table 8, since silver(I) oxide was not able to activate 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl

bromide **(102)**. Seeing that no obvious cause was found for the reoccurring hydrolysis of the donor, $Cu(TFA)_2$ from the supplier was used along with the solid base 5,5'-dimethyl-2,2'-bipyridyl (DMBPY).
Silver(I) oxide is a much less powerful promoter than silver(I) triflate, and therefore the reactions in Table 8 were conducted at room temperature or higher. Different solvents where investigated again, since hydrolysis was suspected to stem from the low availability of the acceptor, due to the solubility being a continuous issue. In all cases, stirring overnight led to consumption of the acceptor with the exception of the glycosylation carried out in CH_2Cl_2 (Entry 3, Table 8), which was stirred for 5 days. In none of the experiments could the disaccharide products be isolated, but rather a mixture of the hydrolyzed donor and the 1,2-benzoyl migration product were isolated in approximately 50-80%.

Table 8 Glycosylation with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide.



Entry	Solvent	Temperature	Additive
1	THF	rt	None
2	MeCN	rt	None
3[a]	CH_2Cl_2	rt	None
4	DME	50°C	3Å MS
5	DME	50°C	Lutidine, 3Å MS

Condition: Acceptor (0.37 mmol), $Cu(TFA)_2$ (0.40 mmol), solvent (7 ml), silver(I) oxide (0.55 mmol), DMBPY (0.28 mmol) and Donor (0.55 mmol). [a]5d

A reaction stirred without copper and base (results not shown) was done for comparison and resulted in an inseparable mixture. However, NMR experiments of the complex mixtures isolated did not reveal the hydrolyzed donor generally observed in the experiments above, but

instead anomeric carbons correlating with disaccharides were found. Yet, the mixture could not be separated and no conclusive identification could be obtained.

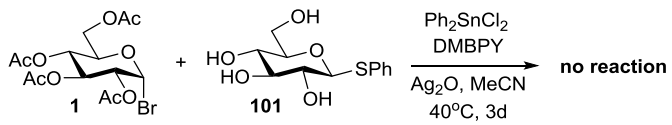
Dong *et al.*[155] achieved regioselective acetylation of either the equatorial 2 or 3 position in methyl α-D-galactopyranoside with protected 6-position by use of a copper catalyst with different ligands in good yields. 1,2-*Cis* diols were necessary to achieve the regioselectivity. This work[155] was conducted in a glovebox, where the other work[149,150,152–154] with copper examined did not state such conditions. Anyhow, copper complexes are known to be hygroscopic and Suthers *et al.*[152] noted the importance of the complex being freshly prepared, and therefore general storage and handling are likely insufficient.

Schuerch *et al.*[149,244] did regioselective alkylation on vicinal hydroxyl groups of different pyranosides by treatment of the sugars with sodium hydride followed by addition of anhydrous copper(II) chloride and lastly adding the alkyl halide. They theorized that copper would deactivate both hydroxyl groups, although the more acidic of two hydroxyl groups would bind more tightly to the copper cation, and hence be less nucleophilic.[149,150] Also, they found that alkyl iodides were vastly superior in the reaction with these chelates and the necessity for the high reactivity of the iodides was ascribed to the deactivation of the hydroxyl groups. Our donor, being less reactive than the alkyl iodides, likely explains the lack of any disaccharide formation in our experiments, and therefore the solubility of the acceptor and the deactivation of the hydroxyl groups would make glycosylation hereof difficult. Since the formation of any disaccharide was not observed, this line of experiments was not further pursued.

3.4 Tin

Tin-mediated manipulations of glucopyranosides and regioselective glycosylation of unprotected carbohydrates have been investigated extensively.

As described in the introduction, Muramatsu and Yoshimatsu[167] were successful in making a tin-mediated regioselective 1,3-linkage between methyl α-D-mannopyranoside **(104)** and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **(1)** in 99% yield by employing 10 mol% of an organotin compound. The 1,3-linked coupling product was of the outmost interest, since it is in line with the previous work of our group.[166,175] As a continuation of the previous work[166] with tin-mediated glycosylation, unprotected phenyl 1-thio-β-D-glucopyranoside **(101)** was chosen as the glycosyl acceptor. Phenyl 1-thio-β-D-glucopyranoside **(101)** was treated with the literature conditions and the glycosyl donor shown in Scheme 33. The conversion of the acceptor was reluctant and TLC indicated decomposition of the donor **1**, and therefore the reaction was terminated after 72 hours. Recovery of any disaccharide products was unsuccessful and instead phenyl 1-thio-β-D-glucopyranoside **(101)** was recovered in 77% yield.

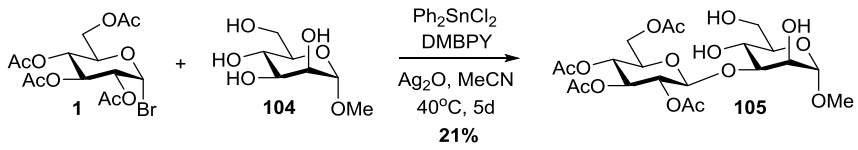


Scheme 69 Application of literature conditions[245] employing acceptor **101**.

Circumventing the decomposition of the glycosyl donor was accomplished by conducting the reaction at room temperature. Nonetheless the product was not recovered from the reaction. The major compound recovered was the donor and the hydrolysis product hereof. The absence of the disaccharide formation was partly ascribed to the lack of 1,2-*cis* vicinal hydroxyl groups in the acceptor, contrary to the α-D-mannopyranosides employed in literature. Thus, an attempt with phenyl 1-thio-β-D-

galactopyranoside **(106)** was made. No major product was detected on TLC, but rather several small spots were observed of which two were possible to isolate. The isolated spots contained several compounds, but disappointingly no disaccharide product.

In the previous work,[166] the combination of organotin compounds and phenyl 1-thio-D-glycopyranoside acceptors have performed highly satisfactory. Therefore interference of the sulphur with the glycosylation was not considered an issue. However, to exclude it entirely, methyl α-D-mannopyranoside **(104)** applied as the glycosyl acceptor, as shown in Scheme 70.



Scheme 70 Application of acceptor **104** with literature conditions.[245]

The glycosylation in Scheme 70 was reluctant to reach completion, and therefore was allowed to stir for 5 days. Purification of the reaction mixture proved difficult and mixtures of products were obtained exclusively. However, the 1,3-linked product **105** could be identified and the yield was calculated from NMR. By employing stoichiometric amounts of the organotin compound the yield dropped to 10%.

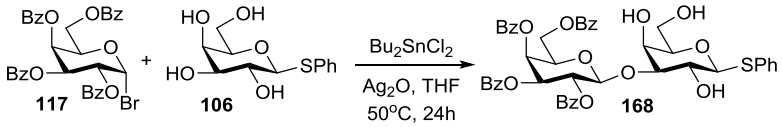
Considering that the line of experiments did not result in the desired product in good yield, another setup was pursued. The main factors considered problematic in the setup above was solubility of the acceptor and stability of the donor. Therefore 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide **(117)** was utilized as the donor instead.

Demailly *et al.*[246] stated that the coordination of acetonitrile to the stannylene acetal could further enhance the nucleophilicity. However, better regioselectivity was observed in chloroform, but neither are ideal solvents for the unprotected carbohydrates. Therefore, THF was chosen as the solvent to increase the solubility of the acceptor.

Muramatsu and Yoshimatsu were unsuccessful in employing Bu_2SnCl_2, but based on previous successes[166] with dibutyltin oxide, it was employed as the organotin reagent in the setup in Table 9.

In Table 9, phenyl 1-thio-β-D-galactopyranoside **(106)** is solubilized in THF and treated with 10 mol% of Bu_2SnCl_2, followed by addition of the glycosyl donor **117**, silver(I) oxide and a pyridine type base. 2,3,4,6-Tetra-*O*-benzoyl-α-D-galactopyranosyl bromide **(117)** is a highly disarmed donor, and therefore the mixture was heated to increase the rate of conversion and full consumption of the starting material according to TLC was observed after 24 hours.

Table 9 Investigations of dibutyltin dichloride mediated regioselective glycosylation.

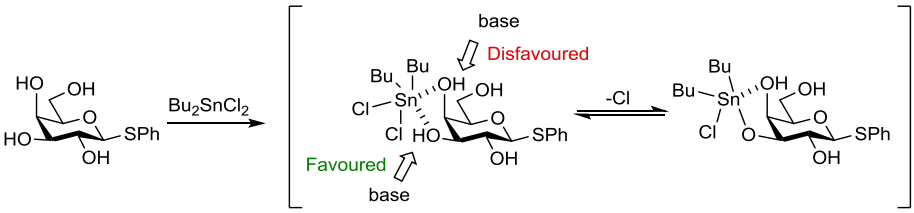


Entry	Conc. (M)	Additive	Yield[a]
1	0.07		19%
2	0.07	Lutidine	51%
3[b]	0.07	Lutidine	26%[c]
4[d]	0.07		<36%[e]
5[f]	0.18	Collidine	-
6[g]	0.14	Collidine	-
7[h,i]	0.12	Lutidine	50%
8[h,j]	0.12	Lutidine	35%

Conditions: Acceptor (0.55 mmol), Bu_2SnCl_2 (0.06 mmol), THF (8 ml), 50°C, Donor (0.83 mmol), base (0.83 mmol) and Ag_2O (0.83 mmol) overnight. [a]Isolated yield, [b]Phenyl 1-thio-β-D-glucopyranoside, [c]1,6-linked product, [d]Ag_2CO_3, [e]orthoester product in complex mixture, [f]4h, [g]60°C, [h]2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl bromide, [i]rt, [j]6h

The setup in Table 9 without additives resulted in the 1,3-linked product **168** in 19% yield (Entry 1, Table 9). Formation of the 1,3-linkage indicated participation of the organotin compound. Interestingly, addition of base to the reaction increased formation of the 1,3-linked product **168** significantly, resulting in 51% yield (Entry 2, Table 9).

The regioselectivity might be explained by the coordination of the organotin reagent with *cis*-diol moiety, and hereby increasing the acidity of hydroxyl groups. This would allow a weak base to facilitate a faster glycosylation by inducing deprotonation of the 3 position, as shown in Scheme 71.



Scheme 71 Assumed mechanism for regioselectivity in the presence of 1,2-*cis* vicinal diol.

The importance of 1,2-*cis* hydroxyl groups were demonstrated in entry GL-142 (Table 9). Phenyl 1-thio-β-D-glucopyranoside **(101)** was submitted to the identical conditions from entry 2 (Table 9), but instead resulting in the formation of the 1,6-linked product **169** in modest yield (26%).

In the previous work done in the group,[166] treatment with base led to formation of the corresponding orthoester product. The experiments conducted here did not adhere to this behavior, but the orthoester product was observed in a complex mixture in one attempt when utilizing silver(I) carbonate to activate the glycosyl donor without the presence of base (Entry 4, Table 9). Silver(I) carbonate was not further examined, due to the undesired formation of the orthoester product **170**.

The influence of the concentration in the reaction was studied in entry 5 (Table 9), where the concentration was 0.18 M in the reaction mixture instead of 0.07 M. The mixture became highly viscous within 4 hours to a point where stirring was disrupted. In the earlier experiments, a slight

thickening of the reactions mixtures had been observed, but in the more concentrated reaction mixture a significant impairment of the reaction occurred. At the concentration 0.14 M thickening of the reaction mixture proved slightly less disruptive, but it was still a highly viscous mixture and the only product observed after 24 hours was the donor, indicating that the high viscosity was inhibiting activation of the donor. The rise in viscosity was ascribed to polymerization of THF.[175]

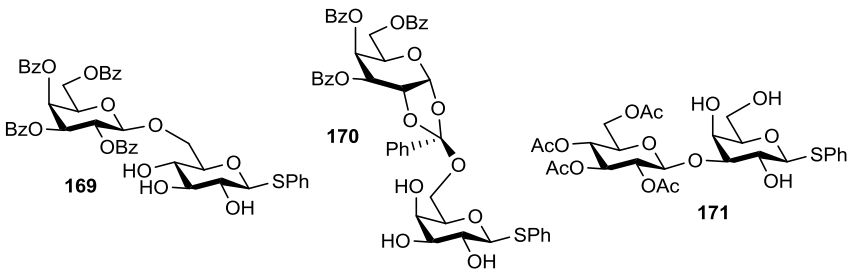


Figure 14 Additional products obtained in Table 9.

An identical reaction to entry 2 (Table 9) was conducted and stirred for 4 days (results not shown). The reaction was followed by TLC, which showed formation of the 1,3-linked product within 24 hours, and hereafter increasing amounts of the hydrolyzed donor was observed, which ended in the disappearance of the 1,3-linked product from TLC. These observations indicate that formation of the desired product was impaired by the thickening of the reaction mixture and decomposition of the product occured. Other solvents able to solubilize unprotected pyranoside acceptors should be considered.

The temperature was assumed to be a significant factor in the increased viscosity of the reactions mixture. To mitigate this effect, an experiement was done by conducting one reaction at room temperature. In entry 7 (Table 9), the reaction was carried out at room temperature with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **(1)** as the donor, since it is more reactive than 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide **(102)**. Full conversion of the starting materials was achieved after 24 hours resulting in the 1,3-linked product **171**, which was isolated in 50%

yield. Raising the temperature to 50°C resulted in faster conversion of the starting materials, but with a significant trade off in the yield (Entry 8, Table 9).

Further investigations regarding tin mediated regioselective glycosylation were not conducted within the time frame of this thesis. Tin mediated regioselective glycosylation has been studied in great length. Generally, the presence of 1,2-*cis* diol moieties play an important role. Furthermore, the activation conditions seem to play a significant role. In Table 9, the 1,3-linked product was obtained with silver(I) oxide as promoter, whereas Maggi and Madsen[166] utilizing the same substrates with Bu_2SnO and silver(I) triflate, achivied formation of the 1,6-linkage product instead. Further investigation into the influence of the solvent, base and temperature could be done to optimize the reaction, along with a substrate scope.

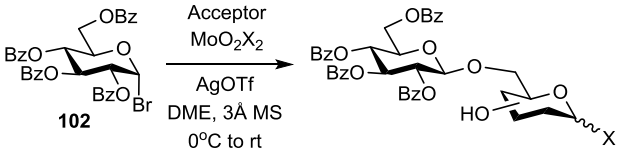
3.5 Molybdenum

Molybdenum was considered a possibility for mediating the regioselective glycosylation, inspired by the protecting group manipulations with molybdenum complexes.[158,159] These transformations demonstrated the ability of $MoO_2(acac)_2$ and $MoCl_5$ in the acylation of monosaccharides containing 1,2-*cis* vicinal hydroxyl groups with increased regioselectivity (section 1.4).

Based on the literature[158,159], a setup using different molybdenum complexes was constructed to investigate the regioselective glycosylation of unprotected glycopyranoside substrates as shown in Table 10 and the products obtained are shown in Figure 15. The previous work in our group[166,168,175] regarding regioselective glycosylations was conducted using unprotected thioglycoside acceptors. Therefore, phenyl 1-thio-β-D-glucopyranoside **(101)** was explored in this setup, and furthermore phenyl 1-thio-α-D-mannopyranoside **(164)** was investigated to examine the regioselective effect of the 1,2-*cis* diol moieties present.

In entry 1 (Table 10) a glycosylation, which employs similar conditions to monobenzoylation of Evtushenko[159], is presented. Here, collidine and dioxane were employed together with $MoO_2(acac)_2$, but 33% of the donor was recovered, along with hydrolysis product of the glycosyl donor. It was assumed, that the base was interfering with the acidic activation conditions. Therefore, collidine was excluded in entry 2 (Table 10). Several spots on TLC made the reaction difficult to examine, and therefore the reaction was terminated after 6 hours, which resulted in 32% yield of the 1,6-linked product **103**, 24% of the donor and 36% of the acceptor. Seeing that both donor and acceptor were recovered from the glycosylation (2, Table 10), an attempt was made by adding more AgOTf to the glycosylation in entry 3 (Table 10). Nonetheless, the yield did not increase significantly.

Table 10 Investigation of molybdenum in metal-mediated glycosylation



Entry	Acceptor	X	Time (h)	Product	Yield[a]
1[b,c,d]	**101**	A	3	-	0%
2[b,c]	**101**	A	6	**103**	32%
3[b,d]	**101**	A	6	**103**	35%
4	**101**	A	3	**103**	45%
5	**101**	B	1.5	**103**	33%
6[e]	**101**	A	0.5	**172**	[f]
7	**164**	A	2	**173**	30%
8	**163**	A	2	**174**	32%
9	**163**	A	3	**174**	13%
10[g,h]	**163**	A	3	**175**	12%
11[i]	**164**	A	2	**173**	0%
12[j]	**165**	A	4	**176**	12%
13[k]	**164**	A	2	**173**	15%
14	**166**	A	1.5	**177**	40%
15	**101**	C	3	**103**	52%
16	**164**	C	3	**173**	35%
17	**163**	C	3	**174**	36%

Condition: Acceptor (1.0 equiv.), Donor (1.3 equiv.), MoO_2X_2 (1,1 equiv., A = acac, B = Cl, C = 2,2,6,6-tetramethyl-3,5-heptanedionate), AgOTf (2.0 equiv.) DME, 3Å MS, 0°C to rt. [a]Isolated yield, [b]dioxane + 4Å MS, [c]rt, [d]additional 0.4 equiv. AgOTf, [e]collidine (1.0 equiv.), [f]complex mixture, [g]2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl bromide and Ag_2O, [h]1,3-linked product, [i]no MS, [j]1,4-linked product, [k]Acceptor and complex heated to reflux in methanol prior to glycosylation.

Phenyl 1-thio-β-D-glucopyranoside **(101)** was glycosylated for 3 hours using DME instead of dioxane as the solvent under the conditions in entry 4 (Table 10) with $MoO_2(acac)_2$. As expected, due to the lack of 1,2-*cis* hydroxyl groups present in the acceptor, the major product was the 1,6-linked product **103**, which was isolated in 45% yield.

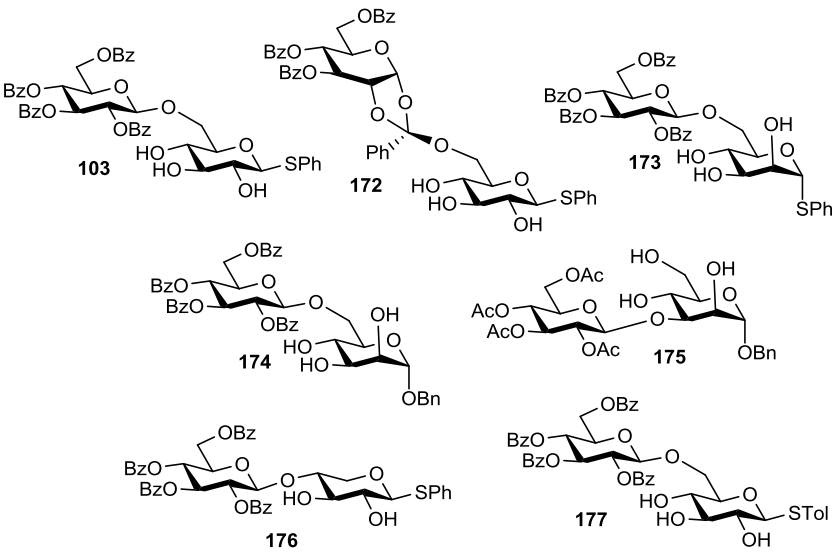


Figure 15 Products obtained during in the investigations in Table 10.

In entry 5 (Table 10), MoO_2Cl_2 was explored under the same conditions, but conversion of the acceptor was slow and the reaction time significantly prolonged. The 1,6-linked product **103** was isolated in 33% yield, and thus further investigations with MoO_2Cl_2 were dismissed.

One attempt using collidine in combination with $MoO_2(acac)_2$ in DME (Entry 6, Table 10) was carried out, resulting in the 1,6-linked orthoester product **172** as an inseparable mixture together with an impurity arising from acetylacetone.

The nature of the anomeric position could influence the interaction between the metal and the sugar. Therefore, in combination with $MoO_2(acac)_2$ both phenyl 1-thio-α-D-mannopyranoside **(164)** (Entry 7, Table 10) and benzyl α-D-mannopyranoside **(163)** (Entry 8, Table 10) were investigated. Full conversion of the acceptors were observed after 2

hours, and 30% and 32%, respectively, of the 1,6-linked products were isolated, which indicates insignificant influence from the anomeric substituent of the acceptor. Nonetheless, yields were moderate compared to the glucose counterpart, which suggests a significant impact on the regioselectivity from the nature of the secondary hydroxyl groups. The results from entry 8 (Table 10) can be compared to the identical glycosylation of benzyl α-D-mannopyranoside **(163)** without a metal complex yielding 13% disaccharide (Entry 9, Table 10), which shows that the molybdenum complex does facilitate regioselective glycosylation of the acceptor in some manner.

The setup was investigated with silver(I) oxide as a neutral promoter and, to compensate for it being a less efficient promoter compared to silver(I) triflate, a more reactive donor was chosen. 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide **(1)** was employed as the donor together with benzyl α-D-mannopyranoside **(163)** as the acceptor (Entry 10, Table 10), resulting in only 12% isolated disaccharide product along with 48% of the hydrolyzed donor. However, the isolated disaccharide product **175** was 1,3-linked, which indicates that the regioselectivity is significantly influenced by the glycosylation conditions.

An experiment without molecular sieves was carried out to establish the importance of the drying agent (Entry 11, Table 10), but it was terminated after 2 hours upon seeing that the hydrolyzed donor was the only significant product according to TLC.

Phenyl 1-thio-β-D-xylopyranoside **(105)** was employed to investigate the regioselectivity without the presence of primary or 1,2-*cis* vicinal hydroxyl groups (Entry 12, Table 10). The glycosylation resulted in the 1,4-linked product **176** in 12% yield, and consequently no convincing regioselectivity for this type of substrates could be established.

Solubility of the acceptor remained a continuous issue, which was limiting the choice of solvents and conditions immensely. Some attempts to circumvent this obstacle were made, where one was to reflux the acceptor with metal complex, hoping to form a complex similar to stannylene

acetal, since stannylene acetals are much more soluble. Phenyl 1-thio-α-D-mannopyranoside **(164)** and $MoO_2(acac)_2$ in methanol were heated to reflux and stirred for 2 hours upon which the solvent was removed and the residue dried in vacuo overnight (Entry 13, Table 10). The resulting residue was submitted to the glycosylation conditions in Table 10. However, the usual milky yellow color of these glycosylations was not observed. Rather a bluish/green color was present, which has previously been noted at the contact of methanol and $MoO_2(acac)_2$, consequently indicating a reaction between methanol and $MoO_2(acac)_2$ instead. The 1,6-linked product was isolated in 15% yield, furthermore indicating an insufficient interaction between the complex and the carbohydrate acceptor. The identical reaction with no complex resulted in 13% yield (Entry 9, Table 10).

Tolyl thioglycosides are known to be more soluble than their phenyl thioglycosides, and therefore *p*-tolyl 1-thio-β-D-glucopyranoside **(166)** was employed as the acceptor in entry 14 (Table 10). The glycosylation resulted in 40% yield of the 1,6-linked product **177**, which indicates that solubility might be a less significant issue.

Molybdenum(VI) dioxide bis(2,2,6,6-tetramethyl-3,5-heptanedionate) was explored in entry 15, 16 and 17 (Table 10), due to its similar nature to $MoO_2(acac)_2$. In all three cases, a slight increase in yield was observed compared to identical reactions with $MoO_2(acac)_2$. The reactions were closely monitored by TLC. According to TLC, no hydrolyzed donor was observed in the reaction mixture. Nonetheless, a significant amount of hydrolyzed donor was isolated upon purification of the reaction.

Application of molybdenum complexes in the glycosylation did induce a regioselective formation of the 1,6-linked product for the unprotected hexopyranosides. However, the presence of 1,2-*cis* diol moieties seemed to decrease the preference for the primary hydroxyl group. The nature of the anomeric atom did not influence the glycosylation in any way. Application of silver(I) oxide as the promoter resulted in the 1,3-linked product **175** in low yield in entry 10 (Table 10), which indicates a significant impact of

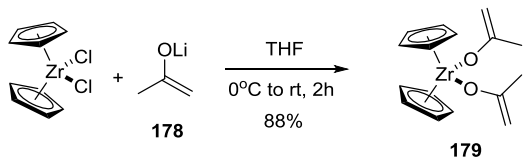
the promoter compared to the 1,6-linked product **174** obtained for the reaction involving silver(I) triflate (Entry 8, Table 10).

3.6 Zirconium

Group 4 metallocenes are known to be oxophilic and complexes with carbohydrates are described in the literature.[247] Therefore, we wanted to investigate any effect zirconium complexes might have on the regioselective glycosylation of unprotected acceptors. First, isolation of a complex between unprotected thioglycosides and zirconium compounds were investigated. This was done in a similar manner to the work of Heck *et al.*[248] Zirconocene dichloride and phenyl 1-thio-β-D-glucopyranoside **(101)** were dissolved in THF, along with Et_3N.

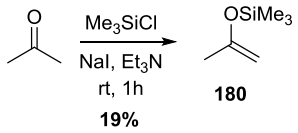
After 24 hours $Et_3NH^+Cl^-$ was filtered off and the filtrate concentrated. The residue was not soluble in either deuterated chloroform or acetone, which the product is and therefore dismissed as the product. Also, NMR studies with phenyl 1-thio-β-D-galactopyranoside **(106)**, zirconocene dichloride and Et_3N in deuterated dichloromethane showed no change in proton peaks over 24 hours. Based on the experiments and the literature[249] the assumption was made that zirconium complexation with carbohydrates was unstable at ambient temperatures. The further pursuit of evidence for any metal and carbohydrate interaction was dismissed, and rather the influence on the regioselectivity in glycosylation was examined.

Erker and co-workers[250] observed complex formation between bis(η-cyclopentadienyl)bis(2-propenolato)zirconium **(179)** and 1,2,3,4-tetra-*O*-methyl-α-D-glucopyranoside. According to the literature[251], bis(η-cyclopentadienyl)bis(2-propenolato)zirconium **(179)** is synthesized from zirconocene dichloride and lithio-2-propenolate **(178)** in THF as shown in Scheme 72.



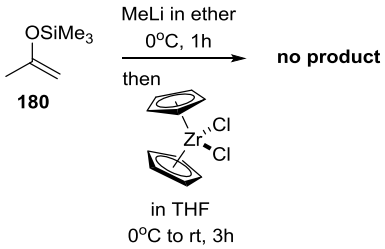
Scheme 72 Literature[251] synthesis of zirconium complex **179**.

To investigate the glycosylation in the presence of bis(η-cyclopentadienyl)bis(2-propenolato)zirconium **(179)**, the ligand was first synthesized according to the literature[252] from acetone, by forming 2-(trimethylsilyloxy)propene **(180)** with trimethylsilyl chloride, as shown in Scheme 73.



Scheme 73 Synthesis of 2-(trimethylsilyloxy)propene **(180)**.

2-(Trimethylsilyloxy)propene **(180)** was treated with methyl lithium to form lithio-2-propenolate[253], to which a solution of zirconocene dichloride was added as shown in Scheme 74, resulting in yellow crystals. However, the presence of bis(η-cyclopentadienyl)bis(2-propenolato)zirconium **(179)**[251] could not be confirmed by ^{1}H NMR in several experiments, and therefore no further attempts to form this complex was made.



Scheme 74 Attempted synthesis of zirconium complex **179**.

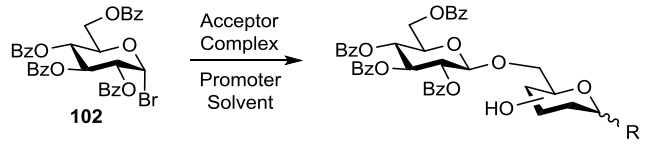
Commercially available zirconium complexes were considered instead and applied in the setup in Table 11. The first experiments conducted were under neutral conditions with silver(I) oxide as the promoter. An investigation into the influence of 1,2-*cis* vicinal hydroxyl groups was done by employing both methyl α-D-glucopyranoside **(69)** and methyl α-D-mannopyranoside **(104)** as acceptors. THF was chosen as the solvent, since it was assumed to stabilize zirconium complexes.[247]

In the setup in Table 11, the acceptor was glycosylated with the disarmed donor in the presence of a zirconium complex. In entries 1 and 2 (Table

11), $Zr(acac)_4$ was used as the complex and the reactions were stirred for 72 hours. In the case of methyl α-D-mannopyranoside **(104)** (Entry 1, Table 11) no conversion to any product was observed. For methyl α-D-glucopyranoside **(69)** (Entry 2, Table 11), an inseparable mixture of mono- and disaccharides was isolated. The complex mixture was accounting for less than 10% yield and therefore not further purified. The complex Cp_2ZrCl_2 was investigated in entry 3 (Table 11) with methyl α-D-mannopyranoside **(104)** as the acceptor, but again no conversion to the product was observed. Instead, the more soluble benzyl α-D-mannopyranoside **(163)** was employed (Entry 4, Table 11), but the major product recovered was donor. Recovery of the donor indicated that it likely was not activated under these conditions. Therefore, 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **(1)** was used as the donor instead in entry 5 (Table 11). Despite the more reactive glycosyl donor, there was still no conversion of the starting materials. In entry 6 (Table 11) the reaction was submitted to reflux instead of room temperature, which led to thickening of THF rather than conversion to the product. Slight thickening of the solvent had been observed in the previous experiments as well, likely caused by polymerization of the solvent.[175] To circumvente this, the solvent was changed to DME (Entry 7, Table 11), but the donor disappeared with no detectable formation of the product, and therefore it was decided to reveret to the more stable 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide **(102)**. DME was kept as the solvent and the molecular sieves used with DME seemed to increase solubility of the acceptor. However, even in this solvent silver(I) oxide was not capable of activating the donor (Entry 8, Table 11).

In entry 9 (Table 11), silver(I) triflate was employed as the promoter instead of silver(I) oxide, upon seeing that DME as a solvent did not restrict the choice of the promoter in the same manner as THF. Using silver(I) triflate as the promoter resulted in the 1,6-linked product **174** in 21% yield after 24 hours.

Table 11 Zirconium mediated glycosylation



Entry	Acceptor	Complex	Promoter	Solvent[a]	Time (h)	Yield[b]
1	**104**	$Zr(acac)_2$	Ag_2O	THF	72	-
2	**69**	$Zr(acac)_2$	Ag_2O	THF	72	-
3	**104**	Cp_2ZrCl_2	Ag_2O	THF	72	-
4	**163**	Cp_2ZrCl_2	Ag_2O	THF	72	-
5[c]	**163**	Cp_2ZrCl_2	Ag_2O	THF	24	-
6[c,d]	**163**	Cp_2ZrCl_2	Ag_2O	THF	24	-
7[c,e]	**163**	Cp_2ZrCl_2	Ag_2O	DME	3	-
8	**163**	Cp_2ZrCl_2	Ag_2O	DME	24	-
9	**163**	Cp_2ZrCl_2	AgOTf	DME	24	21%
10	**163**	Cp_2ZrCl_2	AgOTf	DME	6	40%
11	**163**	$Zr(acac)_4$	AgOTf	DME	4	32%
12	**164**	Cp_2ZrCl_2	AgOTf	DME	6	-
13	**164**	$Zr(acac)_4$	AgOTf	DME	4	11%
14	**101**	Cp_2ZrCl_2	AgOTf	DME	3	30%
15	**101**	Zr(acac)4	AgOTf	DME	2	30%

Conditions: Acceptor (0.37 mmol), zirconium complex (0.41 mmol), Donor (0.48 mmol), promoter (0.74 mmol), solvent, rt. [a]3Å MS with DME [b]Isolated yield, [c]2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl bromide, [d]reflux, [e]50°C.

The reaction was repeated, which revealed that conversion of the staring materials ceased after 5 hours, and hence the reaction was supplied with additional promoter and the reaction time was extended by 1 hour (Entry 10, Table 11). A significant improvement was found with the extra addition of the promoter, which resulted in the 1,6-linked product **174** in 40% yield.

The identical reaction to entry 10 (Table 11) with $Zr(acac)_4$ resulted 32% yield (Entry 11, Table 11). In comparison, this reaction without metal complexes resulted in only 13% yield (Entry 9, Table 10), and we therefore conclude that some degree of regioselectivity could be induced by employing zirconium complexes with benzyl α-D-mannopyranoside **(163)**.

Equivalent reactions to entries 10 and 11 (Table 11) were conducted with the acceptor phenyl 1-thio-α-D-mannopyranoside **(164)**. Surprisingly, the employment of Cp_2ZrCl_2 did not result in any conversion of the donor within 5 hours, even with additional promoter (Entry 12, Table 11). A similar result was obtained by employing $Zr(acac)_4$, which gave rise the 1,6-linked product **173** in 11% yield (Entry 13, Table 11). These results indicate interference with the zirconium complex in the presence of sulfur.

Phenyl 1-thio-β-D-glucopyranoside **(101)** was glycosylated by employing either Cp_2ZrCl_2 (Entry 14, Table 11) or $Zr(acac)_4$ (Entry 15, Table 11). Both reactions resulted in the 1,6-linked product **103** in 30% yield. These results are comparable to the same glycosylation without metal complexes,[166] and hence no regioselective control could be attributed to the zirconium complexes in this case.

Suzuki *et al.*[254] had previously used Cp_2ZrCl_2 in combination with $AgBF_4$ to activate glycosyl fluorides. $AgBF_4$ has also been used to activate several different thioglycosides and glycosyl halides.[255,256] Literature indicates that Cp_2ZrCl_2 and $AgBF_4$ react with each other to form $Cp_2Zr(BF_4)_2$, which in turn decomposes to Cp_2ZrF_2. Cp_2ZrCl_2 takes part in similar rapid reactions with other silver species, such as AgOTf and $AgClO_4$ resulting in $Cp_2Zr(OTf)_2$ and Cp_2ZrFCl, respectively.[257]

The glycosylations requiring additional promoter indicates a reaction between the promoter and the complex. Furthermore, only little regioselectivity was demonstrated and therefore zirconium complexes as regioselective mediators were not further pursued.

3.7 Concluding Remarks

Copper, tin, molybdenum and zirconium complexes were investigated as possible mediators for regioselective glycosylation of unprotected carbohydrate acceptors.

I was not able to obtain any disaccharide product using copper complexes in the glycosylations, which is likely due to the hygroscopic nature of copper. Also, the zirconium complexes applied in our setup did not exhibit much promise in the regioselective glycosylation.

Tin and molybdenum, on the contrary, did exhibit the ability to induce regioselectivity in the glycosylations performed in this work. Furthermore, the regioselectivity was sensitive towards the promoter applied and the presence of 1,2-*cis* vicinal hydroxyl groups.

3.8 Experimental

All solvents were of analytical HPLC grade and reagents were used without further purification as obtained from Sigma-Aldrich, unless otherwise noted. Anhydrous solvents were obtained from the PureSolv™ system unless otherwise stated. Reactions were conducted under argon or a nitrogen atmosphere. Drying of organic layers was done with $MgSO_4$. TLC was performed on aluminum plates coated with silica gel 60. Visualization was carried out by UV and Cerium Sulfate stain. Flash column chromatography was performed with silica gel (35–60 µm) and dry column vacuum chromatography (DCVC)[218] was performed with silica gel (15–40 µm). NMR spectra were recorded with Bruker Ascend 400 spectrometer at 400 MHz and 101 MHz. Chemical shifts are measured relative to the residual solvent signal in $CDCl_3$ (δH = 7.26 ppm, δC = 77.0 ppm) or MeOD (δH = 4.87 ppm, δC = 49.0 ppm). Further assignment of ^{1}H and ^{13}C resonances were based on COSY, HSQC and HMBC experiments. HRMS was performed on a Bruker SolariX XR ESI/MALDI-FT-ICR-MS instrument equipped with a 7 T magnet. The instrument was run in the MALDI mode and externally calibrated with sodium trifluoroacetate cluster ions.

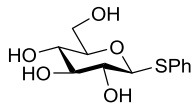
General Procedure A (Tin): The acceptor (0.55 mmol) and dibutyltin chloride (0.06 mmol) were stirred in THF (3 ml) for 10 min at room temperature under inert atmosphere, followed by addition of donor (0.83 mmol), base (0.83 mmol) and silver(I)oxide (0.83 mmol). The mixture was stirred at room temperature or 50°C for 6-24 hours. The reaction mixture was filtered and evaporated, and then purified by column chromatography (4:1 Toluene/Acetone).

General Procedure B (Molybdenum): The acceptor (0.37 mmol) was stirred in dry DME (3 ml) with 3Å MS under an argon atmosphere at room temperature, along with the molybdenum complex (0.41 mmol). The mixture was cooled to 0°C and stirred for 1 h. The donor (0.48 mmol) and the promoter (0.55 mmol) were added, hereby turning the reaction mixture milky and the mixture was stirred for 1-24 hours. The reaction mixture was filtered and the solvent evaporated. The crude was purified by column chromatography (0-3% MeOH in CH_2Cl_2).

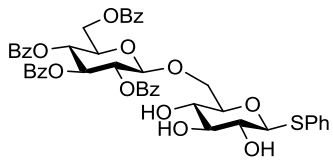
General Procedure C (Zirconium): The acceptor (0.37 mmol) and the zirconium complex (0.41 mmol) were stirred in dry DME (5.0 ml) under a nitrogen atmosphere at room temperature with 3Å MS. The donor (0.48 mmol) was added to the mixture, along with the promoter (0.74 mmol) and the reaction was stirred for 2-24 hours. For some reactions additional of promoter (0.37 mmol) was added. The reaction mixture was filtered

through Celite and the solvent evaporated. The remaining crude was purified by column chromatography (0-3% MeOH in CH_2Cl_2).

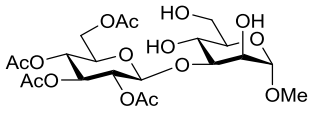
$Cu_2(TFA)_4(THF)_2$: TFAH (1.2 ml, 16 mmol) was added to a blue suspension of $[Cu(OMe)_2]$ (1.0 g, 8.0 mmol) in THF/toluene (1:3, 32 ml) under an argon atmosphere at -35°C. A clear turquoise solution obtained after stirring overnight at -20°C, warming up to 5°C. The solution was evaporated and the crystals where washed with toluene and filtered, affording 1.28 g of turquoise crystals (44%). **IR**[258] 2994w; 2903w; 1687s; 1469w; 1193s; 1150s; 1036w; 879w; 857w; 793w; 730w; 526w; 506w;



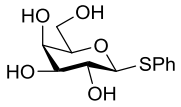
Phenyl 1-thio-β-D-glucopyranoside (101):[259] 0,04 g sodium (1.6 mmol) was dissolved in MeOH (15 ml) followed by addition of phenyl 2,3,4,6-tetra-*O*-acetyl-thio-β-D-glucopyranoside (3.2 mmol). The reaction mixture was stirred overnight, and them neutralized be addition of dry ice, filtered and concentrated. The residue was recrystallized in EtOAc affording the product in 81% yield. **^{1}H NMR**[260] (400 MHz, MeOD) δ 7.60 – 7.50 (m, 2H), 7.36 – 7.19 (m, 3H), 4.59 (d, J = 9.8 Hz, 1H), 3.92 – 3.80 (m, 1H), 3.70 – 3.59 (m, 1H), 3.38 (t, J = 8.7 Hz, 1H), 3.34 – 3.26 (m, 2H), 3.21 (dd, J = 9.7, 8.7 Hz, 1H). **^{13}C NMR** (101 MHz, MeOD) δ 135.6, 132.7, 129.9, 128.3, 89.4, 82.0, 79.6, 73.7, 71.3, 62.8.



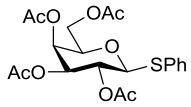
Phenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1,6)-1-thio-β-D-glucopyranoside (103): General procedure B or C. **^{1}H NMR**[166] (400 MHz, $CDCl_3$) δ 8.05 – 8.01 (m, 2H), 7.95 – 7.88 (m, 4H), 7.87 – 7.83 (m, 2H), 7.56 – 7.26 (m, 17H), 5.90 (t, *J* = 9.6 Hz, 1H), 5.72 (t, *J* = 9.7 Hz, 1H), 5.55 (dd, *J* = 9.6, 8.0 Hz, 1H), 4.98 (d, *J* = 7.8 Hz, 1H), 4.72 (dd, *J* = 12.2, 2.9 Hz, 1H), 4.47 (d, *J* = 9.5 Hz, 1H), 4.46 – 4.41 (m, 1H), 4.14 (dd, *J* = 11.8, 2.1 Hz, 1H), 4.11 – 4.08 (m, 1H), 3.91 (dd, *J* = 11.5, 5.4 Hz, 1H), 3.54 – 3.39 (m, 3H), 3.29 (t, *J* = 9.1 Hz, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.5, 165.9, 165.4, 165.3, 133.6-128.2 (aromatic region), 101.4, 87.9, 79.0, 77.8, 72.9, 72.4, 72.1, 72.0, 70.5, 69.7, 69.4, 62.9.



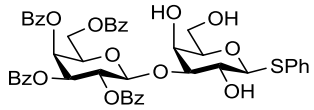
Methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1,3)-α-D-mannopyranoside (105):[167] A solution of methyl α-D-mannopyranoside (0.51 mmol) and diphenyltin dichloride (0.05 mmol) in dry MeCN (10 ml) was stirred for 10 min at room temperature, followed by addition of silver oxide (0.77 mmol), DMBPY (0.39 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide at room temperature. The mixture was heated to 40 °C and stirred for 5 days. The reaction mixture was cooled to room temperature and quenched with a few drops of sat. aq. NH_4Cl, diluted with CH_2Cl_2/acetone (1:1) and filtered to remove insoluble salts. The filtrate was concentrated and the remaining residue was purified by DCVC, resulting in a white solid in 21% yield. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.22 (t, J = 9.6 Hz, 1H), 5.04 – 5.00 (m, 2H), 4.74 (d, J = 1.3 Hz, 1H), 4.61 (d, J = 8.0 Hz, 1H), 4.20 (dd, J = 12.2, 2.6 Hz, 1H), 4.14 (dd, J = 12.2, 6.5 Hz, 1H), 3.87 – 3.72 (m, 5H), 3.72 (dd, J = 9.0, 3.3 Hz, 1H), 3.57 – 3.53 (m, 2H), 3.36 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H).**^{13}C NMR** (101 MHz, $CDCl_3$) δ 170.7, 170.2, 170.0, 169.6, 101.6, 100.5, 84.2, 72.4, 72.2, 71.6, 71.5, 70.0, 68.6, 66.1, 62.4, 62.1, 55.0, 20.8, 20.7 (3xC).



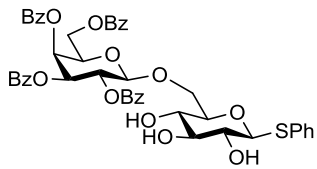
Phenyl 1-thio-β-D-galactopyranoside (106):[261] Compound **167** (68.0 mmol) was dissolved in NaOMe (12 mmol) in MeOH (300 ml) and the solution stirred overnight. However no reaction occurred and therefore sodium (65.0 mmol) in MeOH was added. The reaction was stirred 1 h and neutralized be addition of dry ice, filtered and concentrated. The residue was recrystallized in EtOAc affording the product in 18%. **^{1}H NMR**[262] (400 MHz, MeOD) δ 7.58 – 7.52 (m, 2H), 7.35 – 7.18 (m, 3H), 4.59 (d, J = 9.7 Hz, 1H), 3.90 (d, J = 2.8 Hz, 1H), 3.74 (m, 2H), 3.58 (m, 2H), 3.50 (dd, J = 9.2, 3.3 Hz, 1H). **^{13}C NMR** (101 MHz, MeOD) δ 136.09, 132.09, 129.85, 128.00, 90.30, 80.61, 76.34, 71.01, 70.42, 62.61.



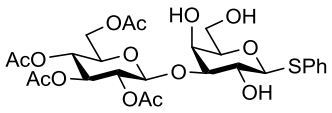
Phenyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranoside (167):[181] Galactose pentaacetate (77.0 mmol) was dissolved in dry CH_2Cl_2 under inert atmosphere followed by addition of thiophenol (177.0 mmol) and BF_3OEt_2 (138 mmol). The mixture is stirred overnight, and then diluted with CH_2Cl_2 and washed aq. sat. NaHCO3. The organic layer was dried and filtered. The solvent was removed *in vacuo* resulting in an oil in 89% yield and the product was used directly in the synthesis of **106**.



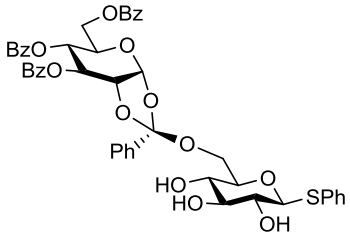
Phenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1,3)-1-thio-β-D-galactopyranoside (168): General Procedure A. **^{1}H NMR**[175] (400 MHz, $CDCl_3$) δ 8.12 – 8.08 (m, 2H), 8.04 – 8.00 (m, 2H), 7.97 – 7.93 (m, 2H), 7.81 – 7.77 (m, 2H), 7.66 – 7.60 (m, 1H), 7.56 – 7.53 (m, 1H), 7.59 – 7.41 (m, 8H), 7.39 – 7.34 (m, 2H), 7.29 – 7.22 (m, 5H), 6.00 (d, J = 3.3 Hz, 1H), 5.78 (dd, J = 10.4, 7.8 Hz, 1H), 5.68 (dd, J = 10.4, 3.4 Hz, 1H), 5.13 (d, J = 7.9 Hz, 1H), 4.63 (dd, J = 11.5, 7.5 Hz, 1H), 4.49 (d, J = 9.8 Hz, 1H), 4.49 – 4.46 (m, 1H), 4.36 – 4.33 (m, 1H), 4.09 (d, J = 2.5 Hz, 1H), 3.89 – 3.78 (m, 2H), 3.68 (dd, J = 8.9, 3.2 Hz, 1H), 3.58 (dd, J = 11.8, 4.3 Hz, 1H), 3.51 – 3.47 (m, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.1, 165.9, 165.7, 165.6, 133.8-127.9 (aromatic region), 102.0, 88.2, 84.1, 78.2, 71.9, 71.3, 70.1, 68.8, 68.3, 68.2, 62.5, 62.4.



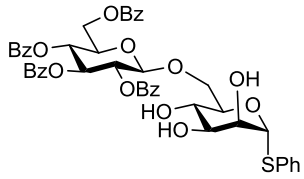
Phenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1,6)-1-thio-β-D-glucopyranoside (169): General Procedure A. **^{1}H NMR**[166] (400 MHz, $CDCl_3$) δ 8.09 – 8.05 (m, 2H), 8.04 – 8.02 (m, 2H), 7.97 – 7.88 (m, 3H), 7.79 (dd, J = 8.3, 1.1 Hz, 2H), 7.70 – 7.13 (m, 16H), 6.00 (d, J = 2.8 Hz, 1H), 5.83 – 5.78 (m, 1H), 5.61 (dd, J = 10.4, 3.4 Hz, 1H), 4.95 (d, J = 8.0 Hz, 1H), 4.68 (dd, J = 11.4, 6.5 Hz, 1H), 4.54 – 4.41 (m, 2H), 4.32 – 4.28 (m, 1H), 4.20 (dd, J = 11.2, 2.5 Hz, 1H), 3.94 (dd, J = 11.2, 4.8 Hz, 1H), 3.55 – 3.43 (m, 3H), 3.26 – 3.20 (m, 1H).**^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.3, 165.7 (2xC), 165.6, 133.8, 133.5 (2xC), 132.8, 132.5, 130.2-128.4, 101.9, 88.1, 78.7, 77.7, 71.8, 71.7, 70.7, 69.9, 69.5, 68.3, 62.1.



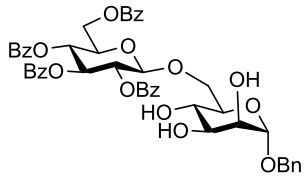
Phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1,3)-1-thio-β-D-galactopyranoside (171): General Procedure A. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.57 – 7.53 (m, 2H, Ar), 7.33 – 7.24 (m, 3H, Ar), 5.25 (t, J = 9.6 Hz, 1H, 3'-H), 5.08 – 4.99 (m, 2H, 2'-H, 4'-H), 4.83 (d, J = 8.0 Hz, 1H, 1'-H), 4.54 (d, J = 9.7 Hz, 1H, 1-H), 4.20 – 4.17 (m, 1H, 6'-H), 4.07 (s, 1H, 4-H), 3.98 (dd, J = 11.6, 6.7 Hz, 1H, 6a-H), 3.84 – 3.77 (m, 2H, 2-H, 6b-H), 3.74 (dt, J = 10.0, 3.9 Hz, 1H, 5'-H), 3.63 (dd, J = 9.0, 3.3 Hz, 1H, 3-H), 3.63 – 3.57 (m, 1H, 5-H), 2.08 (s, 3H, AcO), 2.04 (s, 3H, AcO), 2.03 (s, 3H, AcO), 2.02 (s, 3H, AcO). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 170.8 (C=O), 170.3 (C=O), 170.0 (C=O), 169.5 (C=O), 132.4 (PhS), 132.3 (PhS), 129.2 (PhS), 128.1 (PhS), 101.3 (C-1'), 88.6 (C-1), 83.8 (C-3), 78.3 (C-5), 72.3 (C-3'), 72.1 (C-5'), 71.5 (C-2'), 68.6 (C-4), 68.5 (C-4'), 68.4 (C-2), 62.5 (C-6), 61.9 (C-6'), 20.8 (2xC, AcO), 20.7, (2xC, AcO). **HRMS** (MALDI) calcd for $C_{26}H_{34}O_{14}S$ $[M+Na]^+$ m/z 625.1561 found 625.1590.



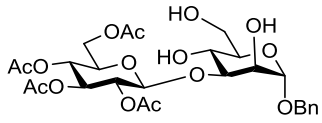
3,4,6-Tri-*O*-benzoyl-1,2-*O*-(phenyl 1-thio-β-D-glucopyranosid-6-yloxy-1-benzylidene)-α-D-glucopyranose (172): General procedure B with $AgCO_3$. **^{1}H NMR**[166] (400 MHz, $CDCl_3$) δ 7.99 – 7.93 (m, 3H), 7.89 – 7.85 (m, 2H), 7.78 (dd, J = 7.9, 1.6 Hz, 2H), 7.60 – 7.03 (m, 18H), 5.99 (d, J = 5.3 Hz, 1H), 5.74 (dd, J = 3.0, 1.2 Hz, 1H), 5.46 (d, J = 8.7 Hz, 1H), 4.82 (ddd, J = 5.2, 3.1, 1.1 Hz, 1H), 4.51 (d, J = 9.7 Hz, 1H), 4.48 (dd, J = 12.7, 3.5 Hz, 1H), 4.35 (dd, J = 12.1, 4.7 Hz, 1H), 4.14 – 4.10 (m, 1H), 3.62 (dd, J = 10.4, 2.3 Hz, 1H), 3.56 – 4.41 (m, 3H), 3.38 – 3.29 (m, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.1, 165.3, 164.5, 134.9, 133.6, 133.07, 130.7-122.1 (aromatic region), 121.0, 97.7, 87.6, 78.2, 78.1, 72.0, 71.7, 70.4, 69.0, 68.5, 67.4, 64.1.



Phenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1,6)-1-thio-α-D-mannopyranoside (173): General Procedure B or C. **^{1}H NMR**[166] (400 MHz, $CDCl_3$) δ 8.14 – 8.10 (m, 2H), 8.05 – 7.97 (m, 4H), 7.93 – 7.89 (m, 2H), 7.86 – 7.82 (m, 2H), 7.64 – 7.25 (m, 15H), 5.93 (t, J = 9.6 Hz, 1H), 5.70 (t, J = 9.7 Hz, 1H), 5.56 (dd, J = 9.7, 7.9 Hz, 1H), 5.53 (d, J = 0.8 Hz, 1H, H-1), 4.95 (d, J = 7.8 Hz, 1H, H-1'), 4.68 (dd, J = 12.2, 3.1 Hz, 1H), 4.48 (dd, J = 12.2, 4.9 Hz, 1H), 4.22 – 4.09 (m, 4H), 3.98 (dd, J = 11.1, 3.9 Hz, 1H), 3.77 – 3.70 (m, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.4, 165.9, 165.6, 165.3, 133.9-127.5 (aromatic region), 101.8, 87.9, 72.7, 72.6, 72.2 (2xC), 72.1, 71.8, 69.6, 69.2, 68.6, 63.0.

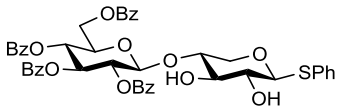


Benzyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1,6)-α-D-mannopyranoside (174): General Procedure B or C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.14 – 8.10 (m, 2H, Ar), 8.07 – 8.03 (m, 2H, Ar), 7.98 – 7.92 (m, 4H, Ar), 7.87 – 7.83 (m, 2H, Ar), 7.63 – 7.59 (m, 1H, Ar), 7.55 – 7.21 (m, 14H, Ar), 5.98 (t, J = 9.7 Hz, 1H, 3'-H), 5.75 (t, J = 9.7 Hz, 1H, 4'-H), 5.63 (dd, J = 9.7, 7.9 Hz, 1H, 2'-H), 5.00 (d, J = 7.8 Hz, 1H, 1'-H), 4.80 (d, J = 1.1 Hz, 1H, 1-H), 4.69 (dd, J = 12.2, 3.1 Hz, 1H, 6a'-H), 4.55 – 4.48 (m, 2H, 6b'-H, $PhCH_2O$), 4.28 – 4.16 (m, 3H, 5'-H, 6a-H, $PhCH_2O$), 3.92 – 3.75 (m, 4H, 2-H, 4-H, 6b-H, 3-H), 3.69 (t, J = 9.5 Hz, 1H, 5-H).**^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.4 (C=O), 165.9 (C=O), 165.6 (C=O), 165.3 (C=O), 137.0 (Ar), 133.5-127.8 (aromatic region), 101.9 (C-1'), 98.8 (C-1), 72.9 (C-3'), 72.4 (C-5'), 72.1 (C-2'), 71.8 (C-3), 71.6 (C-4), 70.6 (C-2), 69.68 (C-6), 69.66 (C-4'), 69.1 ($PhCH_2O$), 68.3 (C-5), 63.0 (C-6'). **HRMS** (MALDI) calcd for $C_{47}H_{44}O_{15}$ $[M+Na]^+$ m/z 871.2572 found 871.2632.

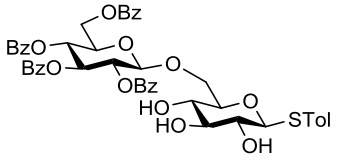


Benzyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1,3)-α-D-mannopyranoside (175): General Procedure B: **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.39 – 7.28 (m, 5H, Ar), 5.22 (t, J = 9.6 Hz, 1H, 3'-H), 5.06 – 5.00 (m, 2H, 2'-H, 4'-H), 4.96 (d, J = 1.1 Hz, 1H, 1-H),

4.71 (d, J = 11.5 Hz, 1H, $PhCH_2O$), 4.61 (d, J = 8.0 Hz, 1H, 1'-H), 4.49 (d, J = 11.5 Hz, 1H, $PhCH_2O$), 4.22 (dd, J = 12.2, 2.5 Hz, 1H, 6a'-H), 4.13 (dd, J = 12.3, 6.6 Hz, 1H, 6b'-H), 3.96 – 3.85 (m, 4H, 2-H, 6-H, 5-H), 3.83 – 3.74 (m, 2H, 5'-H, 3-H), 3.70 – 3.65 (m, 1H, 4-H), 2.08 (s, 3H, AcO), 2.06 (s, 3H, AcO), 2.03 (s, 3H, AcO), 2.01 (s, 3H, AcO).**^{13}C NMR** (101 MHz, $CDCl_3$) δ 170.7 (C=O), 170.2 (C=O), 169.9 (C=O), 169.5 (C=O), 136.8 (Ar), 128.7 (Ar), 128.5 (Ar), 128.4 (Ar), 101.6 (C-1'), 98.6 (C-1), 84.4 (C-3), 72.3 (C-5'), 72.2 (C-3'), 71.9 (C-4), 71.6 (C-2'), 70.2 (C-2), 69.6 ($PhCH_2O$), 68.6 (C-4'), 66.4 (C-5), 62.6 (C-6), 62.1 (C-6'), 20.9 (AcO), 20.7 (3xC, AcO). **HRMS** (MALDI) calcd for $C_{27}H_{36}O_{15}$ $[M+Na]^+$ m/z 623.1946 found 623.1971.

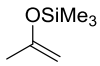


Phenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1,4)-1-thio-β-D-xylopyranoside (GL-176): General procedure B. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.07 (dd, J = 8.3, 1.2 Hz, 2H, Ar), 7.93 – 7.88 (m, 4H, Ar), 7.83 – 7.79 (m, 2H, Ar), 7.44 (m, 12H, Ar), 7.25 (m, 5H, Ar), 5.89 (t, J = 9.7 Hz, 1H, 3'-H), 5.65 (t, J = 9.8 Hz, 1H, 4'-H), 5.51 – 5.46 (m, 1H, 2'-H), 4.90 (d, J = 8.0 Hz, 1H, 1'-H), 4.79 (dd, J = 12.3, 2.6 Hz, 1H, 6a'-H), 4.46 (d, J = 9.4 Hz, 1H, 1-H), 4.37 (dd, J = 12.3, 5.6 Hz, 1H, 6b'-H), 4.20 (ddd, J = 9.4, 5.1, 2.6 Hz, 1H, 5'-H), 3.78 (dd, J = 11.9, 4.2 Hz, 1H, 5a-H), 3.64 – 3.59 (m, 2H, 3-H, 4-H), 3.36 (t, J = 8.4 Hz, 1H, 2-H), 3.23 – 3.17 (m, 1H, 5b-H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.3 (C=O), 165.8 (C=O), 165.2 (C=O), 165.1 (C=O), 133.8, 133.7, 133.5 (2xC), 133.0, 132.0, 130.10-128.22 (aromatic region), 101.9 (C-1'), 88.1 (C-1), 80.8 (C-4), 76.2 (C-3), 73.0 (C-5'), 72.5 (C-3'), 71.8 (C-2'), 71.7 (C-2), 69.2 (C-4'), 67.0 (C-5), 62.6 (C-6'). **HRMS** (MALDI) calcd for $C_{45}H_{40}O_{13}S$ $[M+Na]^+$ m/z 843.2082 found 843.2132.



Tolyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1,6)-1-thio-β-D-glucopyranoside (177): General Procedure B **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.06 – 8.02 (m, 2H, Ar), 7.95 – 7.89 (m, 4H, Ar), 7.84 – 7.80 (m, 2H, Ar), 7.63 – 7.25 (m, 14H, Ar), 7.12 (m, 3H, Ar), 5.91 (t, J = 9.6 Hz, 1H, 3'-H), 5.69 (t, J = 9.7 Hz, 1H, 4'-H), 5.53 (dd, J = 9.7, 7.9 Hz, 1H, 2'-H), 4.98 (d, J = 7.9 Hz, 1H, 1'-H), 4.71 (dd, J = 12.2, 3.0 Hz, 1H, 6a'-H), 4.45 (dd, J = 12.2, 4.9 Hz, 1H, 6b'-H), 4.36 (d, J = 9.7 Hz, 1H, 1-H), 4.16 – 4.10 (m, 2H, 5'-H, , 6a-H), 3.94 – 3.90 (m, 1H, 6b-H), 3.51 – 3.42 (m, 3H, 3-H, 4-H, 5-H), 3.26 – 3.20 (m, 1H,

2-H), 2.32 (s, 3H, $PhCH_3$). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.4 (C=O), 165.9 (C=O), 165.4 (C=O), 165.3 (C=O), 138.7 (Ar, *ipso*), 133.8(Ar, *ipso*), 133.6 (Ar, *ipso*), 133.5 (2xC, Ar, *ipso*), 133.4 (Ar, *ipso*), 130.3-127.8 (aromatic region), 101.6 (C-1'), 88.2 (C-1), 78.7 (C-5), 77.8 (C-3), 72.9 (C-3'), 72.6 (C-5'), 72.0 (C-2'), 71.7 (C-3), 70.8 (C-4), 69.6 (C-4'), 69.5 (C-6), 62.9 (C-6'), 21.3 ($Ph-CH_3$). **HRMS** (MALDI) calcd for $C_{47}H_{44}O_{14}S$ $[M+Na]^+$ m/z 887.2344 found 887.2411.



2-propenyl trimethylsilylether (180):[252] Sodium iodide (62.5 mmol) in MeCN (65 ml) was added dropwise to a solution of acetone (50.0 mmol), Et_3N (62.5 mmol) and trimethylchlorosilane (62.5 mmol) over 15 min at room temperature, when the initial white smoke from mixing the solution disappeared, then precipitation of Et_3NHI salt occurred and the solution became slightly milky and brownish. The stirring was maintained for 30 min. Cold pentane and ice water was added and the aqueous layer was extracted with pentane. The combined organic layer was washed with ice water and aq. NH_4Cl until neutrality, then dried with $NaSO_4$ and the solvent was removed *in vacuo*, hereby affording the product in 19% yield. **^{1}H NMR**[252] (300 MHz, $CDCl_3$) δ 4.07 – 4.03 (m, 2H), 1.77 (s, 3H), 0.23 – 0.19 (m, 9H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 156.1, 91.4, 23.0, 0.3.

4 Identification and Characterization of Glycosyl Transferases Involved in Cell Wall Biosynthesis in Plants

This chapter describes the work conducted during the 3 months external stay at the Joint BioEnergy Institute (JBEI) in Emeryville under the supervision of senior scientist Henrik Vibe Scheller. The purpose of the stay at JBEI was to work towards the identification and characterization of glycosyltransferases involved in the biosynthesis of rhamnogalacturonan I (RGI) contained in pectin. The work presented here is part of the SET4Future Project, which involves partners from academia and industry. The project is aiming to develop new technologies for screening and characterization of enzymes and related biomass substrates. The work was conducted in collaboration with fellow PhD students Faranak Nami and Beatrice Bonora. First, a brief introduction describing polysaccharides of plant cell walls with focus on the structure and biosynthesis of the pectin polysaccharide RGI will be provided and followed by a discussion of the work and results obtained during the external stay.

4.1 Introduction

The polysaccharides found in plant cell walls are a major source of biomass, which is utilized as biofuel, food ingredient, paper, textile, fine chemicals amongst others. The abundant uses of these polysaccharides makes their increased accessibility and modification of outmost interest.[263]

4.1.1. Plant Cell Walls and Polysaccharides

Plant cell walls are the extracellular matrix surrounding plant cells. They posses a highly intricate architecture composed of a complex mixture, mostly made up by polysaccharides and proteins divided into primary and secondary cell walls. The composition of polysaccharides in the cell wall

changes during maturation of a plant. The secondary cell wall is found in the mature plant parts, and consists of up to 70% cellulose and hemicellulose. The primary cell walls are found in developing plant parts, where the turnover and the change in composition of plant polysaccharides in the cell wall is high, in order to accommodate growth and environmental factors.[263–266]

Cellulose, hemicellulose and pectin are the polysaccharides present in plant cell walls. All three polysaccharides are present in both the primary and the secondary cell wall, but pectin is the most abundant in the primary cell wall.[267] These plant polysaccharides provide a rigid physical structure and protect plants against outside environmental factors. Cellulose is the major plant polysaccharide, consisting of a repeating sequence of β-1,4-linked glucose units, and found to be the most abundant in mature plants. Hemicellulose is more complex than cellulose and consists of several different polymers, where xylans are the major constituent.[268] Pectin is not one specific polysaccharide, but a family of complex polysaccharides, which are contained of homogalacturonan (HG), RGI and rhamnogalacturonan II (RGII). Further variations are found in the fine structure, which are linked covalently to the backbone in varying ratios, as presented in Figure 16.[265,267]

So far, no complete pectin structure has been determined, and the sequence of the different domains in pectin are still unknown. Only fractions with different degrees of polymerization have been isolated.[265,267] The knowledge about the sequences of polysaccharides in plant cell walls is thus limited to short polysaccharides of a given type.[263]

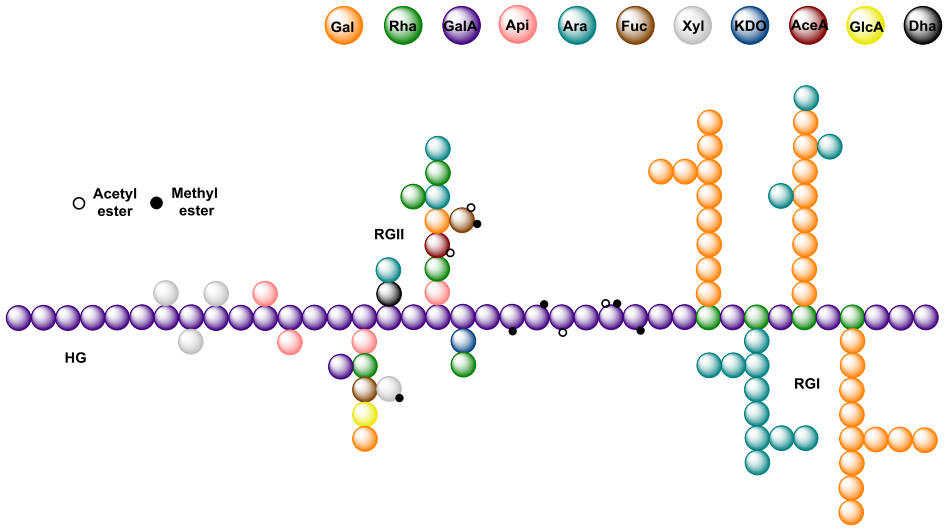


Figure 16 Representation of the pectin structure with covalently linked HG, RGII and RGI.

HG is the major polysaccharide in pectin constituting approximately 65% of the polysaccharide (Figure 16). HG is an unbranched polysaccharide made up by α-1,4-linked galacturonic acids, which to some extend are methylesterified and to a lesser degree acetylated. RGI is present in pectin in varying amounts between 20% and 35%, and, as the only polysaccharide in pectin, it does not have a backbone that consists of only galacturonic acid. The backbone is an alternating sequence of L-rhamnose and D-galacturonic acid. The sugars are linked together with an α-1,4-linkage to D-galacturonic acid and an α-1,2-linkage to rhamnose. Furthermore, the D-galacturonic acids in the backbone are highly acetylated. RGI side chains include α-1,5-linked L-arabinan, β-1,4-linked D-galactan, and β-1,3-linked D-galactan all with further branching. However, the branching and other side chains are more elusive. RGII and variations of HG are the minor plant polysaccharides contained in pectin. RGII has a backbone of α-1,4-linked galacturonic acids with side chains containing 12 different sugars and more than 20 linkages, which makes it the most complex of the plant polysaccharides.[267]

As mentioned above, the function of pectin in the cell wall is mostly mechanical, i.e. providing physical strength and stability to the plant, as

well as protecting against environmental factors, where HG and RGII are considered to be the main contributors to these properties.[269,270] Knowledge of the RGI function is much more scarce. RGI is a structural component of the cell walls and is commonly found more extensively in immature plants. Immature plants require more flexibility, since they are undergoing great alterations. The unesterified regions of HG can interact with cations such as calcium, to form salt bridges between the plant polysaccharides resulting in gel-like properties. The side chains of RGI are providing regulation of the space within closer proximity, hereby limiting calcium ion interaction between the other polysaccharides in pectin, hence preventing the cell wall from becoming rigid.[270–272]

4.1.2. Biosynthesis of Pectin

All living organisms contain polysaccharides which exist in many different forms and configurations. Nature accommodates all these differences, that chemists struggle to produce, such as anomeric configuration, L- and D-forms, furanose and pyranose form, different glycosidic linkages from the anomeric center to any of the other hydroxyl groups, linear and branched glycans and modifications of hydroxyl groups (i.e. ester- and etherfication). Plant cell walls polysaccharides are thought to be synthesized by sequential addition of sugar units onto the non-reducing end of the acceptor molecule by a series of proteins, referred to as glycosyltransferases, in the Golgi apparatus.[273] Pectin biosynthesis is assumed to require a minimum of 67 different transferases, which includes glycosyltransferases, methyltransferases and acetyltransferases.[266]

Pectin is composed of many different sugars, as shown in Figure 16, and various substrates are utilized for the biosynthesis. The donor sugars utilized by glycosyltransferases in the biosynthesis of pectin are the UDP-equivalents of hexoses (D-glucose, D-galactose, D-mannose and L-galactose), 6-deoxy hexoses (L-rhamnose and L-fucose), pentoses (D-xylose, L-arabinopyranose and L-arabinofuranose), hexuronic acids (D-

glucuronic acids and D-galacturonic acid). The acceptor is an oligosaccharide without modifications.[266,273]

The RGI biosynthesis is known to involve uridine diphosphate sugars (UDP) as donors. Therefore, these activated substrates will be the focus onward. UDP-sugars can be found in all living organisms and 30 different types have been identified in plants alone. These UDP-sugars are generated from a few key UDP-sugars via interchanges. Figure 17 gives a small window into the complexity of plant polysaccharide biosynthesis.[266,273]

The interchanges occur via two different mechanisms, interconversion of enzymes and/or the salvage pathway. The enzymes for both the interconversion and the salvage pathway are situated in the cytosol and from there the UDP-sugars are transported into the Golgi apparatus. The salvage pathway recycles sugars from glycans, glycoproteins and -lipids, sugars from structural changes in the cell walls, storage polysaccharides and via photosynthesis from CO_2 to fructose-6-phosphat, which can be further converted to nucleotide sugars.[273]

Sucrose is an important source of sugar in the synthesis of UDP-sugars. It is converted into UDP-glucose by sucrose synthase (SuSy) in the cytosol, which in turn is converted into UDP-rhamnose, UDP-galactose and UDP-glucuronic acid among others. Both UDP-galactose and UDP-glucuronic acid are precursors for several other UDP-sugars as shown in Figure 17. SLOPPY is a promiscuous UDP-sugar phosphorylase, which converts sugar-1-phosphates into the corresponding UDP-sugar (GalA, GlcA, Gal, Xyl, Glc and Ara-1-P). Most of the enzymes involved in the biosynthesis and interconversions of UDP-sugars have been identified and the regulation of UDP-sugars is speculated to have a key role in the synthesis of pectin.[267,273]

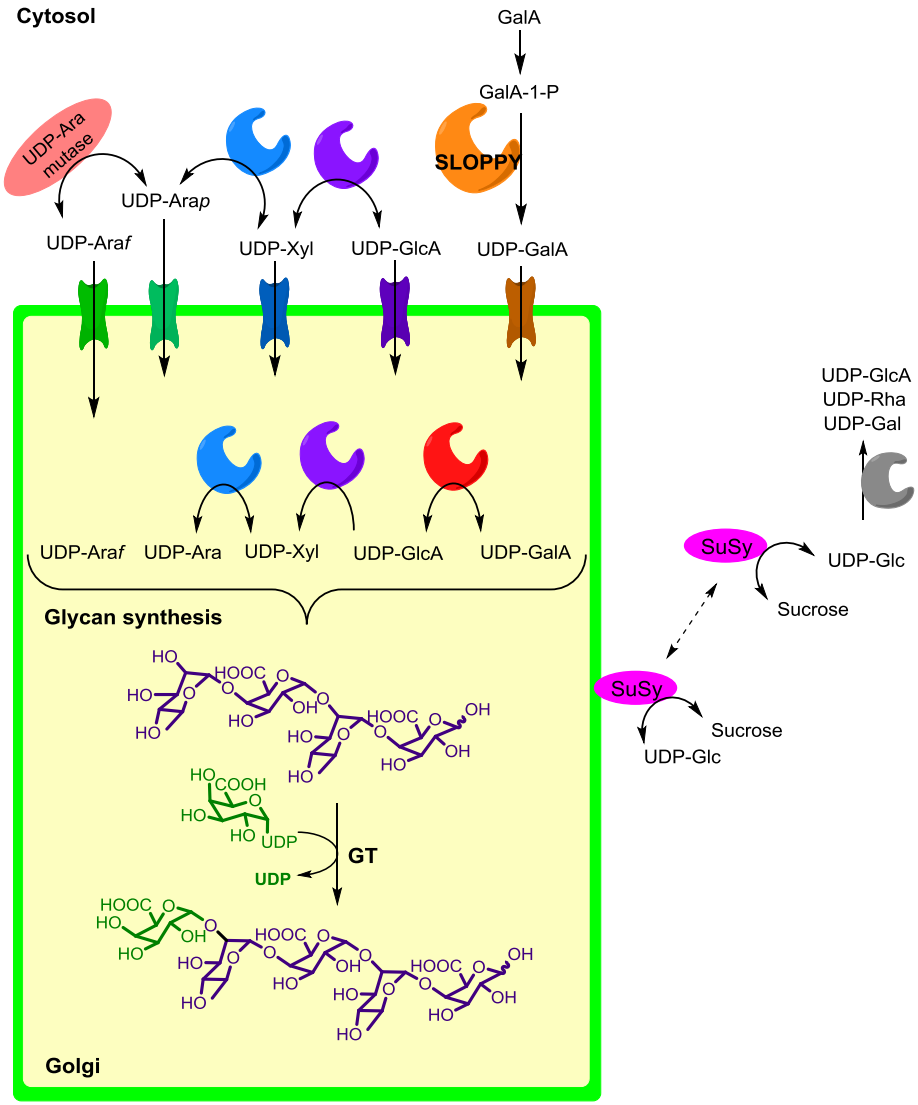


Figure 17 Biosynthesis of glycans depend on interconversion of activated UDP-sugars.

4.2 Aim of the Project

Biomass represents a resource of both energy and food, but due to its properties remains mostly unused. Some different strategies for efficient access to these largely untouched sources of pectin polysaccharides are considered. One idea is to utilize chemical or enzymatic treatment of extracted pectin to obtain the desired properties, while another approach is modulating the biosynthesis of pectin to produce the desired properties.[263,274] In order to modulate the biosynthesis, a better understanding of the reaction mechanism for glycosyltransferases is needed.

The biosynthesis of RGI requires several tranferases to initiate, elongate and provide branching. Several glycosyltransferases in pectin biosynthesis have been characterized in *in vitro* studies. A couple of examples are the glycosyltransferase substituting glucuronic acid onto the xylan backbone[275] and arabinofuranosyltransferases,[276] elongating the 1,5-linked α-L-arabino oligosaccharide. The homogalacturonan backbone is synthesized by elongation of galacturonan and catalyzed by HG:α-1,4-D-galacturononsyl-transferases which transfer galacturonic acid from the corresponding UDP-sugar.[277]

The goal of this project is to identify the glycosyltransferase involved in elongating the RGI backbone by addition of galacturonic acid units through the corresponding UDP-sugar and characterize the activity *in vitro*.

4.3 Results and Discussion

To identify new glycosyltransferases related to the biosynthesis of the RGI backbone, microsomes were prepared from mung beans. Microsome is the general description for small particles consisting of cell membranes extracted from cells by ultracentrifugation. These microsomal membranes contain membrane-bound proteins, such as glycosyltransferases and when extracted they retain enzymatic activity. The enzymatic activity was confirmed by the Bradford protocol.

The initial glycosylsyltransferase activities were investigated in an assay adopted from Konishi[276], using UDP-galacturonic acid to elongate the chemically synthesized RGI hexamer **181**.[278] Chemical synthesis of the acceptor provided the means to obtain a well-defined and pure molecule to investigate the transferase activity. The products generated by the enzymatic reactions were characterized by HPLAEC and mass spectrometry.

4.1.3. Investigation of Glycosyltransferase Activity

An assay adopted from Konishi et al.[276] was used for the initial investigations of rhamnosyltransferases. In this setup, MES-KOH was used as the buffer (pH = 6.5) and the detergent Trition X-100 was used to prevent precipitation of the enzyme, as well as absorption onto surfaces of the wells. Furthermore, manganese chloride was added to increase solubility of the enzyme.

The length of the acceptor had previously been shown to be an important factor with glycosyltransferases.[279] In case of arabinosyltransferase, an increase in enzyme activity using arabinan acceptor from pentamer to octamer could be established.[279] In this initial assay, the acceptor was a short chemically well-defined synthesized RGI backbone fragment **181**, that consists of six units alternating between L-rhamnose and D-galacturonic acid, as shown in Figure 18.[278] UDP-galacturonic acid was used as the substrate to elongate the RGI hexamer **181**.

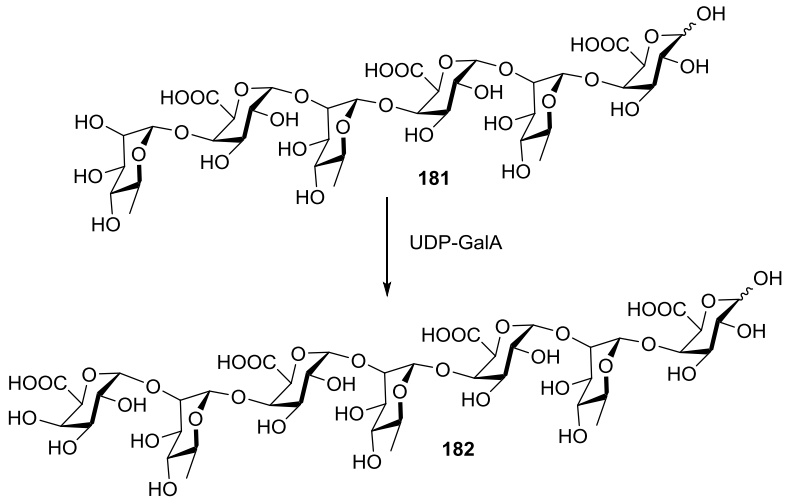


Figure 18 Elongation of chemically synthesized RGI hexamer acceptor by galacturonic acid transferase

A high amount of acceptor **181** was used in the initial experiments to make detection of the desired product **182** possible, and a time frame for the enzymatic activity was established in that same assay, as presented in Figure 19. Based on the assay, it was concluded that enzyme activity had stopped after four hours. The formation of new products was followed with HPLAEC.

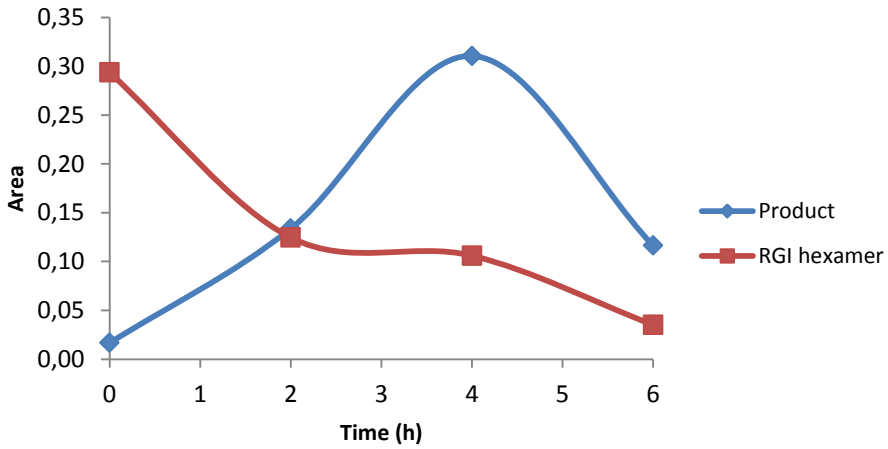


Figure 19 Investigation of time optimum (0-6 hours). Microsomes, RGI hexamer **181**, UDP-GalA, MES-KOH (pH = 6.5), Triton X-100, $MnCl_2$.

Several peaks were observed, and therefore control experiments without either UDP-GalA or the RGI hexamer acceptor **181** were conducted and terminated after four hours. All results were compared to the retention times for the acceptor, UDP-GalA, UDP and UMP. The standards and control experiments revealed only one peak in the full assay, which did not belong to any of them. The peak was collected and subjected to dialysis removing sodium ions present. Analysis by mass spectroscopy revealed $[M + Na]^+ = 1183.3156$, which was in agreement with the calculated mass for the desired product **182**, shown in Figure 18.

4.1.4. Optimization of Assay

To optimize the conditions of the assay, several aspects were considered, which included detergent, pH, different cations, cation concentration, temperature, UDP-GalA dependency, amount of microsome and different additives.

Observation in the initial assays gave way for questions regarding influence of the reaction volume on conversion to the product **182**, which therefore was investigated first. The initial assays were made with a 50 µL reaction volume and for comparison an assay with a reaction volume of 10 µL. The comparison between these two reaction volumes are shown in Figure 20.

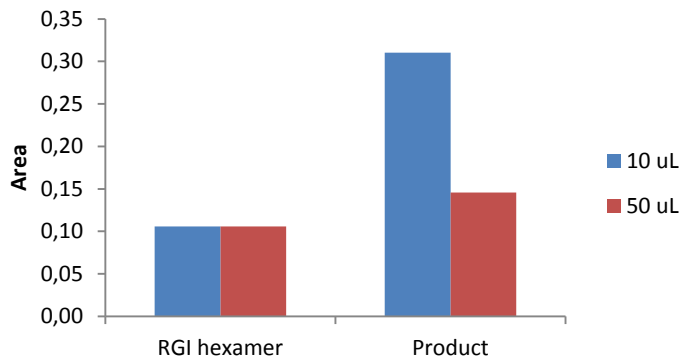


Figure 20 Influence of the reaction volume on product formation. Microsomes, RGI hexamer **181**, UDP-GalA, MES-KOH (pH = 6.5), Triton X-100, $MnCl_2$, 4 h.

Conversion with 10 µL reaction volume occurred much more readily, and after 4 hours the ratio between the acceptor **181** and the product **182** was significantly larger for the 10 µL reaction volume, than the 50 µL reaction volume. This was ascribed to the inaccessibility of the microsomes, which gather at the bottom of the reaction tube. Thus, was the larger reaction volume less homogeneous, than the smaller volume.

One of the tested aspects was the effect of different detergents. In agreement with the in-house experience, three different detergents were chosen, Triton X-100, Nonidet P-40 and CHAPS. CHAPS proved non-compatible with the column and no discernable difference was found between Triton X-100 and Nonidet P-40. Therefore Triton X-100 was chosen for further optimization.

An assay to determine the pH optimum was done using MES, HEPES and TAPS buffers ranging from pH = 5.0 to 9.5. However, due to the technical problems with the HPLC column, detection was difficult. Indications were given that the assay performed better at a higher pH, and therefore assays with HEPES and TAPS were redone at pH = 8.0 and 8.5, as shown in Figure 21.

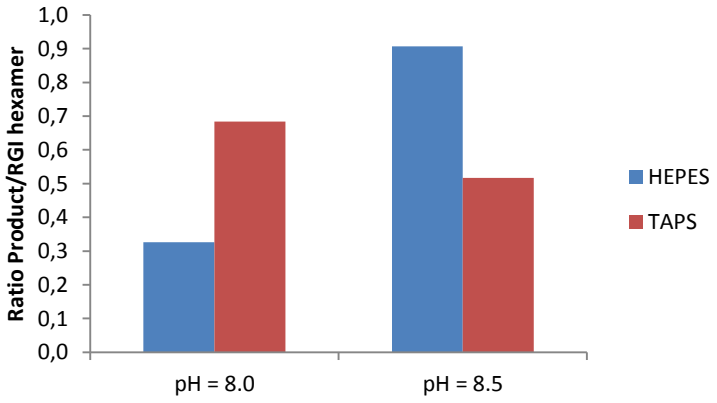


Figure 21 Test of HEPES and TAPS buffers at pH = 8.0 and 8.5. Microsomes, RGI hexamer **181**, UDP-GalA, Triton X-100, $MnCl_2$, 4 h.

The ratio of product **182** versus remaining acceptor **181** after 4 hours showed an optimum at pH = 8.5 employing HEPES. Later a study including MES, HEPES and TAPS was repeated by Beatrice Bonora,[280] which showed that the pH optimum was at 8.0 using HEPES buffer.

One of the functions of the cations present in plants is to facilitate enzyme. Manganese is a commonly applied cation, which was also employed by Konishi *et al.*[276] to facilitate glycosyltransferase activity. Magnesium was considered as an alternative to manganese, due to its application in similar assays[275] and therefore also of interest in this investigation.

Assays for both manganese and magnesium at different concentrations were conducted at pH = 8.5 and terminated after 4 hours, as shown in Figure 22. Enzymatic activity with no addition of a cation can be assigned to manganese present in the homogenization buffer for obtaining the microsomes. Interestingly, the enzymatic activity decreases with increased manganese concentration. On the contrary, magnesium increased the enzymatic activity immensely and an optimum around 2 mM of magnesium ion was established.

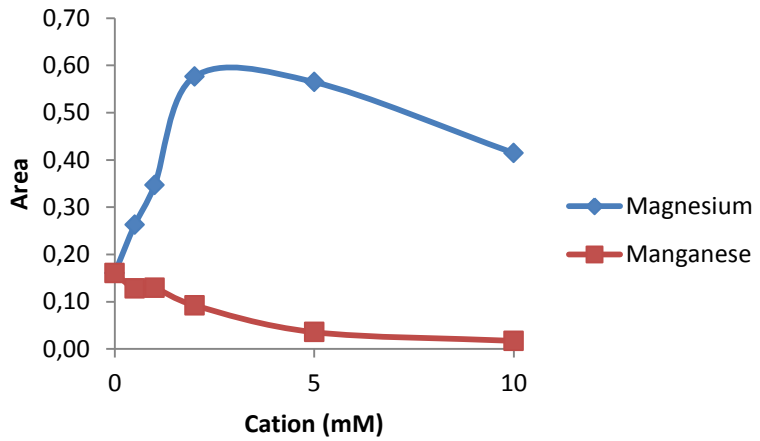


Figure 22 Investigation of the influence of manganese and magnesium concentration on the product formation. Microsomes, RGI hexamer **181**, UDP-GalA, HEPES-KOH (pH = 8.5), Triton X-100, 4 h.

For comparison identical assays at pH = 6.5 were conducted, as shown in Figure 23, where magnesium was again the superior cation. However, the inherent level of manganese supplied a better enzymatic activity than further addition of both cations. For both cations detection was tedious and upon comparing Figure 23 with Figure 22 it is noteworthy, that detection decreased significantly, which indicates better conversion at pH = 8.5.

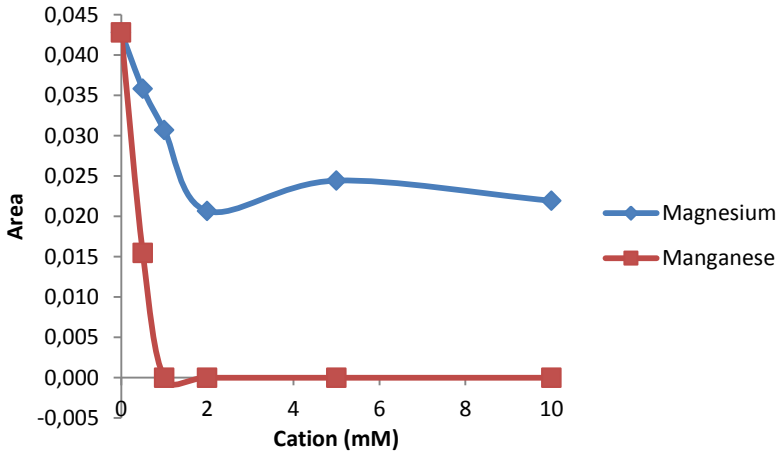


Figure 23 Investigation of the influence of manganese and magnesium concentration on the product formation. Microsomes, RGI hexamer **181**, UDP-GalA, MES-KOH (pH = 6.5), Triton X-100, 4 h.

4.4 Conclusion and Outlook

A glycosyltransferase elongating the RGI backbone was successfully identified in the microsomes obtained from mung beans. The desired heptamer backbone fragment **182** was isolated and confirmed by HRMS. The characteristics of the glycosyltransferase were evaluated in assays investigating the optimum conditions of the enzymatic activity. Optimum for cation and pH were identified, and interestingly it differed from previous results established identified by Scheller and co-workers.[275]

After completing this stay, Beatrice Bonora[280] found a temperature optimum of 35°C compared to the standard conditions conducting the assays at 25°C. The results obtained in this work should be repeated in order to be fully verified, due to many technical problems during the work.

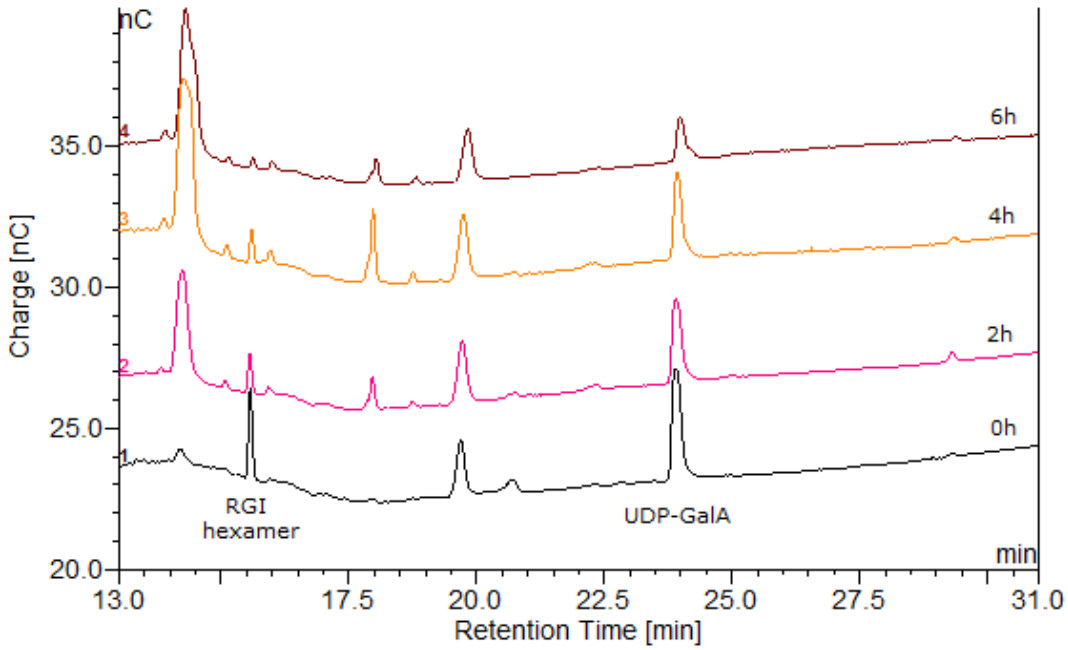
4.5 Experimental

General Assay: All solutions (Final concentration in assay: RGI acceptor (0.1 µM), Buffer (50 mM), detergent (1%v/v), cation (5 mM), microsomes (50 µg), UDP-GalA (1.0 mM) were stored in a bucket of ice until the incubation start. One reaction mixture of 50 µL for each variable (pH, cation, detergent) was made. All components except UDP-GalA were carefully mixed and incubated on ice for 10 minutes. Then UDP-GalA was added and the reaction mixture carefully mixed, then divided into 10 µL reaction volumes in individual tubes and one terminated immediately. The other samples were terminated after 4h at 25°C by addition of 10 µL of water and heating at 95°C for 5 min. The terminated samples were transfered to a VWR centrifugal filter (nylon, 0.45 µM, cat. no. 82031-360) along with 80 µL of water and centrifuged for 5 minutes at 15000 rpm. The samples were stored at 5°C until analyzed with HPLAEC (1000mM NaOAc gradient from 0-85% and constant 100mM NaOH conc., Dionex CarboPac™ PA-200 Column for Oligosaccharide Analysis).

Preparation of Microsomes: The microsomes employed in the assays were prepared from mung beans seeds grown in the dark for 3 days at 27°C. Hereafter all manipulations of the plant material were performed in a cold room (4°C). The Mung Beans were collected and washed, then 1-1.5 cm segments were cut out under the hook of etiolated hypocotyls. The segments were grinded in a mortar in buffer (1ml/g) of 20 mM MES-KOH (pH = 6.5), 0.4 M sucrose, 1 mM dithiothreitol (DTT) and 1 mM phenylmethanesulfonyl fluoride (PMSF, protease inhibitor, 0.1 M in ethanol), then the remaining buffer was added (total buffer 5 ml/g). The suspension was filtered through Miracloth to remove cell debris and centrifuged at 3.000g for 15 min. The supernatant was further ultracentrifuged at 50.000g for 1 hour. The pellets of microsomal membranes were resuspended in the homogenization buffer (20 mM MES-KOH (pH = 6.5), 0.4 M sucrose, 1 mM DTT, 70 µL per gram plant material). The aliquots were frozen in liquid nitrogen and stored in a -80°C freezer. The membranes were determined to contain 7 mg protein per ml.

Determination of time optimum

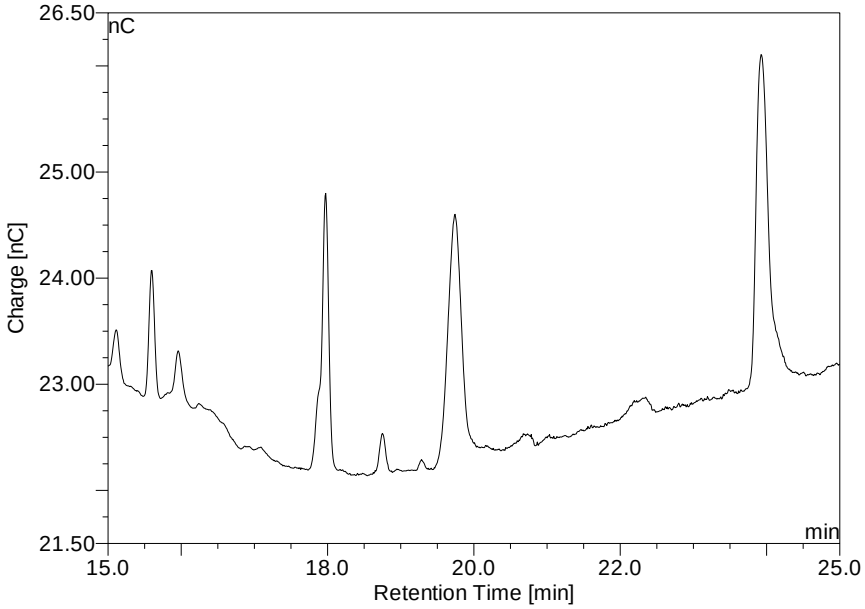
Retention time (Product) ~18.0 min



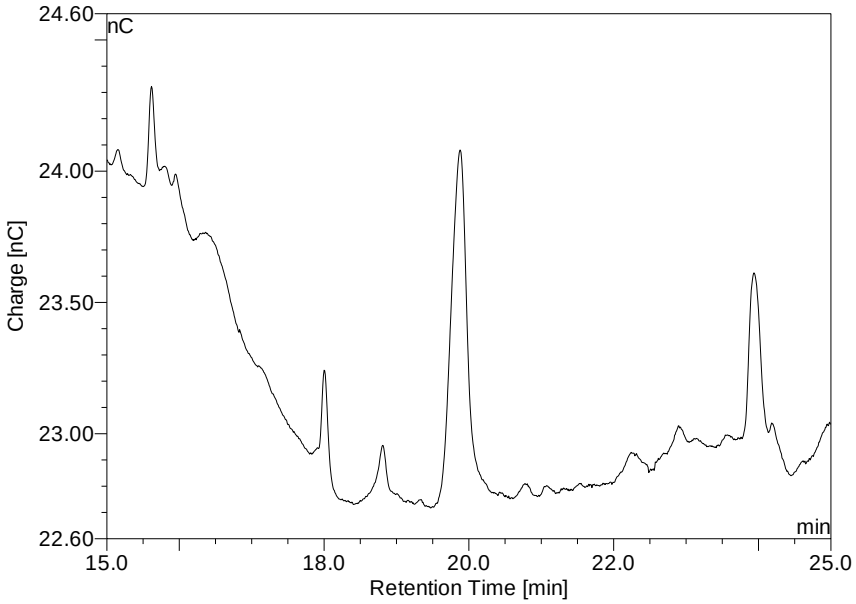
Reaction Volume

Retention time: RGI hexamer ~ 15.6 min and product ~18.0 min

10µL volume (4h):

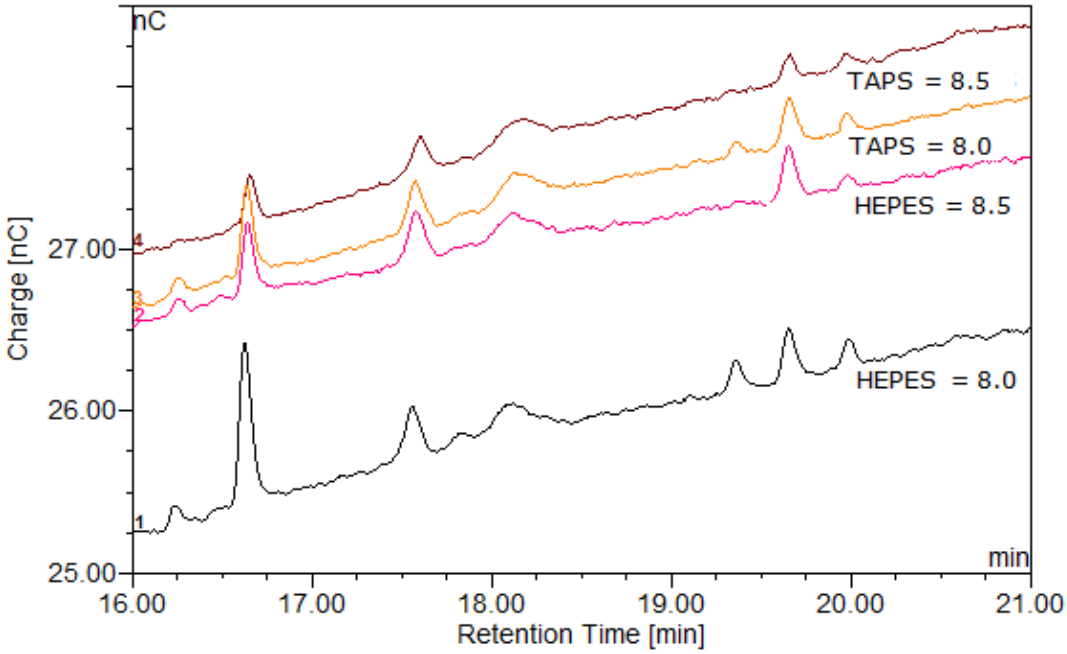


50µL volume (4h):



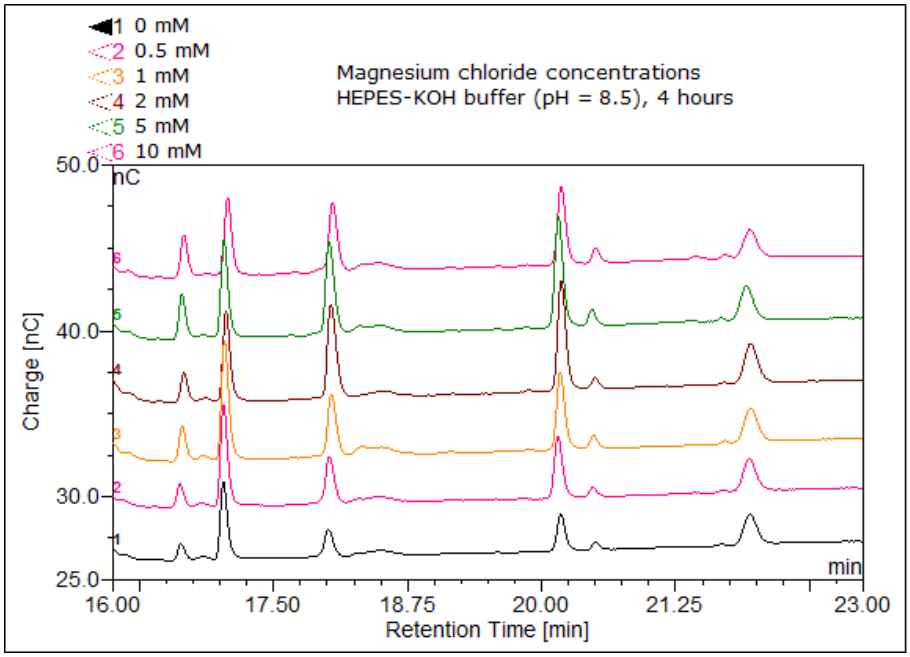
pH investigation

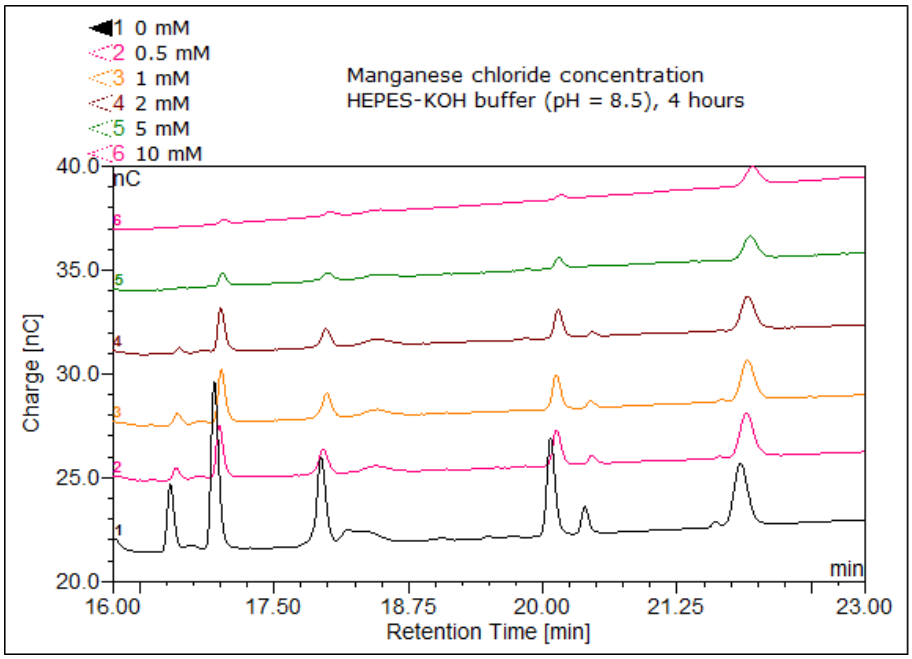
Retention time: RGI hexamer ~ 16.6 min and product ~19.6 min



Cation concentration

Retention time: RGI hexamer ~16.6 min and product ~18.0 min





5 Conclusion

In all the work contained in this thesis, method development of glycosylation reactions has been the focus in order to highlight some of the challenges along with a few possible solutions. Methods generally applicable in carbohydrate synthesis still remain as intangible as stated by Paulsen[107] already in 1982: "… each oligosaccharide synthesis remains an independent problem, whose resolution requires considerable systematic research and a good deal of know-how." However, in this work several tools to improve carbohydrate synthesis were investigated.

One tool for glycosylation involving glycosyl bromides and unreactive acceptors has emerged in the form of a powerful promoter system utilizing iodonium ions, and therefore avoiding the use of heavy metals.

Regioselectivity in the glycosylation of unprotected glycopyranosyl acceptors beyond 1,3- and 1,6-linkages was not obtainable. The regioselectivity for these two linkages was improved by metals-mediated procedures, but the glycosylation conditions and the configuration of the unprotected acceptor have a significant impact on the regioselectivity.

Glycosyl halides are having a renaissance within the field of regioselective glycosylation with activation through silver(I) oxide. However, it is not a powerful promoter and therefore more consideration could be given to manipulation of the reactivity of the unprotected acceptors.

Finally, a glycosyl transferase for the addition of galacturonic acid onto the RGI backbone was identified and partially characterized. This part illustrates the glycosylation reaction in nature and the importance of enzymatic tools for the manipulation of oligosaccharides.

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Publication

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